METHODS AND COMPOSITIONS FOR THE DETECTION OF PREGNANCY IN RUMINANTS

This application claims the benefit of Provisional Application 60/393,615, filed July 03, 2002 the entire contents of which are incorporated by reference.

Pursuant to 35 U.S.C. §202(c), it is acknowledged that the U.S. Government has certain rights in the invention described, which was made in part with funds from the National Institutes of Health, Grant Number NIH HD 32475.

FIELD OF THE INVENTION

This invention relates to the field of molecular biology and reproductive biology. More specifically, the present invention provides materials and methods for rapid and efficient detection of pregnancy in ruminants.

BACKGROUND OF THE INVENTION

Several publications are referenced in this application by author name, year and journal of publication in parentheses in order to more fully describe the state of the art to which this invention pertains. Several patents are also referenced throughout the specification. The disclosure of each of these publications and patent documents is incorporated by reference herein.

Reproductive efficiency (time from calving to conception), feed costs associated with maintaining

non-pregnant cows, annual milk production (dairy) and weaning weights (beef) are major constraints for optimization of management in bovine industries. Accordingly, early and accurate detection of pregnancy are critical to efficient management of cattle. However, currently there is no rapid and reliable bovine pregnancy test.

Human chorionic gonadotropin (Fishel SB, et al. (1984) Science 223:816-8) is present in high amounts, in the urine of pregnant humans, and is the basis for the rapid pregnancy test that is sold commercially. No corresponding chorionic gonadotropin protein has been identified in bovine blood or urine.

There are many methods of determining pregnancy in cows, but they all have some difficulty associated with them. Mechanical methods, which detect actual fetuses, are reasonably accurate, but cannot be conducted early in pregnancy. There are two such methods commonly used. The first is through rectal palpation for presence of the fetus (Sasser RG, et al. (1987) J Reprod Fertil Suppl 34:261-71; Beal WE, et al. (1992) J Anim Sci 70:924-9; Fricke PM (2002) J Dairy Sci 85:1918-26; Hanzen C, et al. (1987) Vet Rec 121:200-2). This method is accurate after 40-50 days of pregnancy. In some cases skilled technicians can determine pregnancy using this method as early as 35 days, but this is not recommended, because manipulation of the uterus and the fetal membranes may cause abortion. The second common method is ultrasound. Ultrasound is accurate as early as day 27 of pregnancy, but also requires a skilled technician (Sasser RG, et al. (1987) J Reprod Fertil Suppl 34:261-71; Beal WE, et al. (1992) J Anim Sci 70:924-9; Fricke PM (2002) J Dairy Sci 85:1918-26; Hanzen C, et al. (1987) Vet Rec 121:200-2). Both of these methods must be performed relatively late following establishment of pregnancy, which occurs

between days 14 to 19 (Thatcher WW, et al. (1995) J Reprod Fertil Suppl 49:15-28; Bazer FW, et al.(1991) J Reprod Fertil Suppl 43:39-47; Helmer SD, et al. (1989) J Reprod Fertil 87:89-101; Bazer FW, et al. (1986) J Reprod Fertil 76:841-50; Thatcher WW, et al.(1986) J Anim Sci 62 Suppl 2:25-46).

Other means of pregnancy testing involve measuring hormone or chemical changes that occur during pregnancy. Early methods were based on detecting the steroid hormone, progesterone. While these techniques still have merit and utility (Booth JM, et al.(1979) Br Vet J 135:478-88; Holdsworth RJ, (1979) Br Vet J 135:470-7; Pengelly J (1979) Vet Rec 104:328; van de Wiel DF, et al. (1978) Tijdschr Diergeneeskd 103:91-103; Macfarlane JS, et al. (1977) Vet Rec 100:565-6; Dobson H, et al. (1976) Br Vet J 132:538-42; Hoffmann B, et al. (1976) Br Vet J 132:469-76), timing of progesterone tests is difficult, and improper timing can lead to an incorrect determination of pregnancy status.

The luteal phase of the estrous cycle is the time between ovulation and luteolysis (characterized by the regression of the corpus luteum). Progesterone is released into the milk, and also circulates in the blood during the luteal phase of the estrous cycle, and during pregnancy. In a well-timed blood test, a low concentration of progesterone is interpreted to reflect a non-pregnant cow that is undergoing luteolysis in preparation for the next estrous cycle. However, it is difficult to distinguish a pregnant cow from a non-pregnant cow in the luteal phase of estrous. This is because if a non-pregnant cow is still in the luteal phase when she is tested, her progesterone levels will be similar to those of a pregnant cow. Missing luteolysis by one or two days contributes to a high rate of false positive test (a cow determined to be pregnant by the

test, but actually being non-pregnant).

One technique to improve the accuracy of progesterone tests involves determining progesterone concentration on the day of artificial insemination and then again three weeks later on day 21. Because most estrous cycles are 16 to 24 days in length with an average of 21 days, then it is possible to sample most non-pregnant cows at a time that progesterone concentration would be low. This helps distinguish the pregnant cows from non-pregnant cows, but still does not provide accurate and reliable results (Sasser RG, et al. (1987) J Reprod Fertil Suppl 34:261-71; Pitcher PM, et al. (1990) J Am Vet Med Assoc 197:1586-90; Oltenacu PA,et al. (1990) J Dairy Sci 73:2826-31; Nebel RL (1988) J Dairy Sci 71:1682-90; Gowan EW, et al. (1982) J Dairy Sci 65:1294-1302).

Another pregnancy specific marker, Early pregnancy factor (EPF) (Ito K, et al.(1998) Am J Reprod Immunol 39:356-61; Cavanagh AC, et al. (1994) Eur J Biochem 222:551-60; Sakonju I, et al. (1993) J Vet Med Sci 55:271-4; Klima F, et al. (1992) J Reprod Immunol 21:57-70) also has been called Early Conception Factor (ECF) (Gandy B, et al. (2001) Theriogenology 56:637-47; Cordoba MC, et al. (2001) J Dairy Sci 84:1884-9; Nancarrow CD, et al. (1981) J Reprod Fertil Suppl 30:191-9) was first described by its ability to inhibit rosette formation between T lymphocytes and red blood cells. This bioassay was used to detect pregnancy in ruminants, but never was developed fully into a useful diagnostic test, because the specific protein that had this unique activity was difficult to purify. More recently, EPF has been shown to be a member of the chaperonin 10 gene family (Cavanagh AC, et al. (1994) Eur J Biochem 222:551-60). However, two recent studies by independent laboratories have shown that EPF is not a

very useful diagnostic for early pregnancy (Gandy B, et al. (2001) Theriogenology 56:637-47; Cordoba MC, et al. (2001) J Dairy Sci 84:1884-9).

Yet another putative pregnancy marker, Pregnancy-Specific Protein B, is reported to be present in binucleate cells of the trophoblast as early as day 21 of pregnancy in cows (Sasser RG, et al. (1986) Biol Reprod 35:936-42; Humblot F, et al. (1988) J Reprod Fertil 83:215-23; Sasser RG, (1989) J Reprod Fertil Suppl 37:109-13; Kiracofe GH, et al. (1993) J Anim Sci 71:2199-205; Szenci O, et al. (1998) Theriogenology 50:77-88). The PSPB is a member of the Pregnancy Associated Glycoprotein or PAG family (Zoli AP, et al.(1992) Biol Reprod 46:83-92; Xie S, et al. (1994) Biol Reprod 51:1145-53; Roberts RM, et al. (1995) Adv Exp Med Biol 362:231-40; Green JA, et al. (2000) Biol Reprod 62:1624-31; Perenyi ZS, et al. (2002) Reprod Domest Anim 37:100-4; Sousa NM, et al. (2002) Reprod Nutr Dev 42:227-41; de Sousa NM, et al. (2003) Theriogenology 59:1131-42; Karen A, et al. (2003) Theriogenology 59:1941-8). Specifically, it is identical to PAG-1 (Xie S, et al. (1994) Biol Reprod 51:1145-53; Roberts RM, et al. (1995) Adv Exp Med Biol 362:231-40). There are now 20 different PAG genes that have been identified (Roberts RM, et al. (1995) Adv Exp Med Biol 362:231-40). However, detection of this protein in blood is not accurate until after day 30 and this protein has a very long half-life, so it remains in circulation for several months following parturition, which limits its utility in post-partum cows. When cows are mated or inseminated prior to 70 days post partum, residual post-partum PSPB concentrations (from previous pregnancy) lowers the accuracy of using PSPB as a marker for pregnancy (Kiracofe GH, et al. (1993) J Anim Sci 71:2199-205; Sasser RG, et al. (1988) J Anim Sci 66:3033-9).

Further research indicates that interferon induced proteins may play a role in pregnancy. During the peri-implantation period, the bovine conceptus secretes interferon (IFN)-tau (τ) (Roberts RM et al., J Reprod Fert 1991; 43:3-12; Thatcher WW, et al., J Reprod Fertil 1995; 49:15-28.). It is generally accepted that IFN-tau is the maternal recognition of pregnancy signal in ruminants. In cattle, IFN-tau functions to limit the release of the luteolysin prostaglandin $F_{2\alpha}$, thereby rescuing the corpus luteum from regression (Thatcher WW, et al., J Reprod Fertil 1995; 49:15-28; Bazer FW, et al., Amer J Reprod Immunol 1997; 37:412-420). In this way, continued exposure of the endometrium to progesterone supports the processes of adhesion, implantation, placentation and embryogenesis and prevents the ensuing estrous cycle. Interferon-τ also induces the expression of numerous uterine proteins. One of these uterine proteins is the ubiquitin homolog, interferon stimulated gene product 17 (ISG17). ISG17 becomes covalently linked to targeted intracellular proteins (Johnson GA, et al., Biol Reprod 1998; 58:898-904), is released from endometrial cells (Austin KJ, et al., Biol Reprod 1996 54:600-606), and may function as a paracrine modulator (Pru JK, et al., Biol Reprod 2000; 63:619-628).

Bovine ISG17, also known as ubiquitin cross-reactive protein (Austin KJ, et al., Biol Reprod 1996 54:600-606;Perry DJ, et al., Mol Endocrinol 1999;13:1197-1206), is the ortholog of human (Blomstrom DC,et al., J Biol Chem 1986;261:8811-8816; Haas AL, et al., J Biol Chem 1987;262:11315-11323) ISG15. The difference in nomenclature is real versus relative mass. The bovine ISG17 gene (Perry DJ, et al., Mol Endocrinol 1999;13:1197-1206) encodes a protein of 17-kDa that migrates to an apparent Mr of 17,000 on PAGE gels. Human and mouse genes encode a pre-ISG15 that is processed

(Knight E Jr et al., J Biol Chem 1988;263:4520-4522; Potter JL et al., J Biol Chem 1999; 274:25061-25068) to yield a mature 17-kDa protein that migrates to an apparent Mr of 15,000 on PAGE gels. ISG15 has been shown to have an extracellular cytokine role in inducing proliferation of natural killer cells and non-major histocompatibility complex-restricted cytotoxicity (D'Cunha J et al., Proc Natl Acad Sci U S A 1996; 93:211-215). Also, ISG15 (Recht M et al., J Immunol 1991; 147:2617-2623) and ISG17 (Pru JK, et al., Biol Reprod 2000; 63:619-628) have been shown to induce release of IFN-γ by cultured peripheral blood mononuclear cells.

Other IFN-induced proteins have been identified which may play an important role in establishing communication between the mother and embryo and in preparing the uterus for implantation. These IFN-induced proteins include granulocyte chemotactic protein 2 (Teixeira MG et al., Endocrine 1997; 6:31-37; Staggs KL, Austin KJ et al., Biol Reprod 1998; 59:293-297), the GTPase Mx (Ott TL et al., Biol Reprod 1998; 59:784-794; Ellinwood NM et al., J Interferon Cytokine Res 1998; 18:745-755), 2',5'-oligoadenylate synthetase (Schmitt RA et al., Biol Reprod 1993; 48:460-466), and the 1-8 family.

Three functional members of the 1-8 gene family have been isolated on a genomic DNA fragment of less than 18 Kb in humans (Lewin AR et al., Eur J Biochem 1991; 199:417-423). These members included 1-8U, 1-8D and Leu-13/9-27. The promoter of each family member contained multiple IFN-stimulated response elements (ISREs) (Reid LE et al., Proc Nat Acad Sci 1989; 86: 840-844). While 1-8U and 1-8D were exclusively induced by type 1 IFNs (α, β, ω), Leu-13 gene expression was promoted by type 1 and type 2 IFNs (Jaffe EA et al., J Immunol 1989; 143:

3961-3966; Chen YX et al., J Immunol 1984; 133: 2496-2501). Leu-13 was originally identified as a 16-kDa protein that was localized to the surface of normal T cells (Pumarola-Sune T et al., J Immunol 1986; 137:826-829). Treatment of T cells with anti-Leu-13 monoclonal antibody caused homotypic aggregation of these cells (Pumarola-Sune T et al., J Immunol 1986; 137:826-829). Leu-13 also has been immunolocalized to adult human endothelium of major organs and to epithelium of renal proximal tubules, cervix and esophagus (Pumarola-Sune T et al., J Immunol 1986; 137:826-829). From these observations it was concluded that the 1-8 proteins modulate cellular growth and adhesion.

Accordingly, it has been determined that there are many genes which are differentially expressed in response to pregnancy, and therefore provide an early and accurate means of determining pregnancy status.

In light of the importance of early and accurate pregnancy determination in efficient cattle production, and the current lack of an early and accurate means of determining pregnancy in cattle, a need exists for the further characterization of genes, which are differentially expressed in pregnant animals.

SUMMARY OF THE INVENTION

In accordance with the present invention, methods and compositions for detecting ruminant pregnancy are provided. Specifically, pregnancy specific markers are provided, as well as methods of determining ruminant pregnancy by detecting differential expression of the same.

One embodiment of the invention comprises at least one isolated, enriched, or purified nucleic acid molecule which is differentially expressed in pregnant ruminants, or which encodes a pregnancy specific marker. A nucleic

acid molecule encoding a pregnancy specific marker includes any nucleic acid molecule which encodes any protein which is a variant or derivative of a pregnancy specific marker, and which retains pregnancy specific marker function. Exemplary pregnancy specific marker nucleic acid molecules are listed in Tables 2A-B..

Also provided in accordance with the invention are oligonucleotides, including probes and primers, that specifically hybridize with the nucleic acid sequences set forth above.

In a further aspect of the invention, recombinant DNA molecules comprising the nucleic acid molecules set forth above, operably linked to a vector are provided. The invention also encompasses host cells comprising a vector encoding a pregnancy specific marker of the invention.

One embodiment of the invention comprises an isolated, enriched, or purified pregnancy specific marker polypeptide. A pregnancy specific marker polypeptide includes any polypeptide which is a variant or derivative of the pregnancy specific marker and which retains pregnancy specific marker function. Preferably, a pregnancy specific marker polypeptide is a protein encoded by a sequence shown in Tables 2A-B.

In another aspect of the invention, an antibody immunologically specific for a pregnancy specific marker polypeptide is provided. Such antibodies may be monoclonal or polyclonal, and include recombinant, chimerized, humanized, antigen binding fragments of such antibodies, and anti-idiotypic antibodies. Preferably, these antibodies specifically recognize a protein encoded by a sequence shown in Tables 2A-B.

In another aspect of the invention, methods for detecting pregnancy specific marker molecules in a biological sample are provided. Such molecules can be

pregnancy specific marker nucleic acids, such as mRNA, DNA, cDNA, or pregnancy specific marker polypeptides or fragments thereof. Exemplary methods comprise mRNA analysis, for example by RT-PCR. Immunological methods include for example contacting a sample with a detectably labeled antibody immunologically specific for a pregnancy specific marker polypeptide and determining the presence of the polypeptide as a function of the amount of detectably labeled antibody bound by the sample relative to control cells. In a preferred embodiment, these assays may be used to detect a sequence as set forth in Tables 2A-B, or a protein encoded by the same.

In a further aspect of the invention, kits for detection of ruminant pregnancy are provided. An exemplary kit comprises a pregnancy specific marker protein, polynucleotide, or antibody, which is optionally linked to a detectable label. The kits may also include a pharmaceutically acceptable carrier and/or excipient, a suitable container, a solid support, and instructions for use.

BRIEF DESCRIPTION OF THE DRAWINGS

The following drawings form part of the present specification and are included to further demonstrate certain embodiments. These embodiments may be better understood by reference to one or more of these drawings in combination with the detailed description of specific embodiments presented herein.

Figure 1 illustrates exemplary cDNA synthesis and RsaI digestion. The lanes contain the following: Lane 1- 1 kb Marker, lane 2 PRT: Tester cDNA (SMART PCR: 18 cycles), lane 3 -PRD: Driver cDNA (SMART PCR: 18 cycles), lane 4 - RsaI digested PRT, lane 5 RsaI digested PRD, lane 6 - 1 kb Marker.

Figure 2 illustrates an exemplary PCR-Select subtraction procedure. PCR-Select cDNA subtraction was performed in both directions and subtracted cDNA libraries were prepared. Subtractive hybridization was performed with 1 (tester - PRT): 30 (driver - PRD) ratio in both directions and subtracted cDNA pool was amplified by PCR. Primary PCR samples were given 27 cycles and secondary PCR samples were given 10 cycles. Lanes contained the following: lane 1 - 1 kb Marker, lane 2 unsubtracted PRT, lane 3 - unsubtracted PRD, lane 4 - PRT primary PCR, lane 5 - PRD primary PCR, lane 6 - PRT secondary PCR, lane 7 - PRD secondary PCR and lane 8 - 1 kb marker.

Figures 3A-B illustrate exemplary screening of subtracted libraries. 2 µl of each PCR-amplified insert (100 ng) was arrayed in 96-well format on a nylon membrane. Two identical membranes were prepared for each 96-well plate. Each membrane was hybridized with a different P-32 labeled cDNA probe. The probe was either (1) a subtracted PRT-specific cDNA probe or (2) a subtracted PRD-specific cDNA probe. In figure 3a, one plate from Driver-Specific (PRD) subtracted library was subjected to differential screening analysis using the tester-specific (PRT) and driver specific (PRD)-subtracted probes. In figure 3b, six plates from Tester - Specific (PRD) subtracted library was subjected to differential screening analysis using Tester-Specific (PRT) and Driver-Specific (PRD) subtracted probes.

Figures 4A-B illustrate exemplary virtual northern blot analysis. For virtual northern blot analysis, cDNA was amplified from Tester and Driver total RNA samples using SMART cDNA amplification technology. These cDNAs were run on an agarose gel and transferred to a membrane.

Each membrane was then hybridized with different radioactive cDNA probes randomly selected from differential screening analysis. Virtual northern blot analysis on more than twenty different clones from each subtracted library was performed. Six are shown here. The lanes contain the following: lane 1 SMART amplified cDNA from PRT total RNA and lane 2 SMART amplified cDNA from PRD total RNA. All clones described herein were confirmed to be differentially expressed in whole blood mRNA from day 18 pregnant cows when compared to non-pregnant cows using virtual northern blot analysis (not shown). Figure 4a illustrates exemplary clones from PRT-specific subtracted libraries. Figure 4b illustrates exemplary clones from PRD-specific subtracted libraries.

Figure 5 is a picture of the lateral flow system used for detecting ISG17 in blood from pregnant cows. The control line is on the left and the test line is on the right. The sample flows laterally by capillary action past immobilized gold-conjugated antibody. The pregnancy-associated antigen is detected by the formation of a visible complex. Samples 831 and 117 are positive (from pregnant cows) and Samples 87 and 1747 are negative (from non-pregnant cows).

Figure 6 is a chart showing the clones which are differentially expressed in pregnant bovines.

DETAILED DESCRIPTION OF THE INVENTION

In accordance with the present invention, compositions and methods are provided for the detection of pregnancy in ruminant animals, preferably bovines. A series of nucleic acid sequences which are upregulated in response to pregnancy, and proteins encoded thereby, are used to advantage in a variety of assays as pregnancy

specific markers for the rapid and efficient differentiation between pregnant and non-pregnant animals. These sequences are provided in Tables 2A-B.

Suitable assays for pregnancy detection include, without limitation, PCR, nucleic acid hybridization assays (e.g., Northern and Southern blotting), immunoassays (e.g. ELISA, Immunohistochemistry, RNA ELISA), and Western Blotting.

Day 18, following artificial insemination, was chosen for isolation of mRNA molecules associated with pregnancy because by this time, if present, the early embryo should have established its presence, primarily through release of interferon-tau from the trophoblast (Thatcher WW, et al. (1995) J Reprod Fertil Suppl 49:15-28; Roberts RM (1989) Biol Reprod 40:449-52; Roberts RM, et al. (1990) Oxf Rev Reprod Biol 12:147-80; Roberts RM (1991) Bioessays 13:121-6; Demmers KJ, et al. (2001) Reproduction 121:41-9; Mann GE, et al. (2001) Reproduction 121:175-80; Hansen TR, et al. (1999) J Reprod Fertil Suppl 54:329-39). Interferon-tau does not enter the blood stream and is not useful as a marker for pregnancy. However, several genes may be induced by interferon-tau in the uterus and by other conceptus-derived hormones-cytokines. Use of these "surrogate markers" for determining the presence of the embryo is the basis for the present invention.

Several uterine proteins have been shown to be induced by conceptus derived interferon-tau and include, but are not limited to Ubiquitin Cross-Reactive Protein (UCRP; aka ISG15) (Hansen TR, et al. (1999) J Reprod Fertil Suppl 54:329-39; Nighswonger AM, et al. (2000) J Anim Sci 78:1393-4; Johnson GA, et al. (1999) Endocrine 10:243-52; Johnson GA, et al. (1998) Biol Reprod 58:898-904; Austin KJ, et al. (1996) Biol Reprod 54:600-6), the 1-8 family of proteins (Pru JK, et al.

(2001) Biol Reprod 65:1471-80), alpha chemokines (Teixeira MG, et al. (1997) Endocrine 6:31-7), MX-GTPase (Johnson GA, et al. (2002) J Endocrinol 174:R7-R11; Yankey SJ, et al. (2001) J Endocrinol 170:R7-11; Ott TL, et al. (1998) Biol Reprod 59:784-94). However, it was not published until recently (Yankey SJ, et al. (2001) J Endocrinol 170:R7-11) that white blood cells also could be activated by conceptus-derived interferon-tau. This occurred, presumably in the subset of blood cells that were delivered to vascular beds in the uterus, exposed to interferon-tau and then picked up in venous drainage. The pattern of gene expression by white blood cells in response to pregnancy on day 18 is described for the first time herein.

Other products, in addition to interferon-tau are released by the conceptus and could activate specific genomic responses in white blood cells. The hypothesis was tested that white blood cells produced a genomic response to the presence of the early developing blastocyst. This hypothesis was tested using mRNA isolated from whole blood. Whole blood, rather than purified lymphocytes, was processed immediately to purify RNA. This step optimizes the chances for success in profiling gene expression in blood from day 18 pregnant when compared to non-pregnant cows. Any change in gene expression because of purification and manipulation of nucleated cells in the blood was, therefore, eliminated because whole blood was used, and the mRNA pools were immediately stabilized.

The present invention concerns compositions and methods of identification and use of members of a pregnancy-specific cDNA library created on day 18 of pregnancy. In addition, the present invention concerns the protein sequences of the cDNA library. Such cDNA sequences may be inserted into expression vectors in

order to generate recombinant proteins. The present invention also provides antibodies generated against such protein sequences. In certain embodiments, such compositions and methods are of use for pregnancy detection in mammalian species.

I. Definitions

The following definitions are provided to facilitate an understanding of the present invention:

For purposes of the present invention, the term PRT refers to an animal that is positive for pregnancy and PRD refers to an animal that is negative for pregnancy. In addition, in the present invention, the term Tester refers to the PRT sample and Driver refers to the PRD sample.

Moreover, for the purposes of the present invention, "a" or "an" entity refers to one or more of that entity; for example, "a cDNA" refers to one or more cDNA or at least one cDNA. As such, the terms "a" (or "an", "one or more" and "at least one" can be used interchangeably herein. It is also noted that the terms "comprising," "including," and "having" can be used interchangeably. Furthermore, a compound "selected from the group consisting of" refers to one or more of the compounds in the list that follows, including mixtures (i.e. combinations) of two or more of the compounds. According to the present invention, an isolated, or biologically pure molecule is a compound that has been removed from its natural milieu. As such, "isolated" and "biologically pure" do not necessarily reflect the extent to which the compound has been purified. An isolated compound of the present invention can be obtained from its natural source, can be produced using laboratory synthetic techniques or can be produced by any such chemical synthetic route.

"Pregnancy specific marker" is a marker which is differentially expressed in pregnant animals versus non-pregnant animals. "Ruminant pregnancy specific marker" or "bovine pregnancy specific marker" is a marker which is differentially expressed in pregnant ruminants or bovines, compared to non-pregnant ruminants or bovines. "Bovine pregnancy inducible marker" is a marker which is induced, or caused to be expressed directly or indirectly in response to pregnancy. Such markers may include but are not limited to nucleic acids, proteins, or other small molecules.

The term "surrogate marker" of pregnancy is a marker which is directly or indirectly differentially expressed in response to pregnancy. Specifically, a surrogate marker may be any gene expression product which is differentially expressed in pregnant animals. A surrogate marker can be a polynucleotide, a protein, or any gene expression product, but is preferably an mRNA or protein expression product. Preferably, a surrogate marker of pregnancy is one which is differentially expressed in early pregnancy, for example at day 18 of pregnancy.

The term "early pregnancy" refers to a stage of pregnancy where a trophoblast has developed, but the fetuses are not yet detectable by mechanical means, such as ultrasound, radiograph, palpation. Optionally, "early pregnancy" may refer to any time before 4 weeks (e.g. day 15-23). In one embodiment, "early pregnancy" is day 18 of pregnancy.

A "ruminant" is an even-toed, herbivorous, ungulate mammal (Order Artiodactyla) that chews cud (ruminate) and has a complex, usually four-chambered stomach containing micro-organisms that break down cellulose. Ruminants include but are not limited to cattle, sheep, antelope, deer, giraffes, elk, moose, caribou, and yak.

A "detection reagent" or a "marker detection reagent" is any substance which has binding affinity for a pregnancy specific molecule, and includes but is not limited to nucleic acid molecules with sufficient affinity to hybridize to the pregnancy specific marker, probes, primers, antibodies, fragments thereof, and the like. The "detection reagent" or "marker detection reagent" may optionally be detectably labeled.

The term "detectably label" is used herein to refer to any substance whose detection or measurement, either directly or indirectly, by physical or chemical means, is indicative of the presence of the target bioentity in the test sample. Representative examples of useful detectable labels, include, but are not limited to the following: molecules or ions directly or indirectly detectable based on light absorbance, fluorescence, reflectance, light scatter, phosphorescence, or luminescence properties; molecules or ions detectable by their radioactive properties; molecules or ions detectable by their nuclear magnetic resonance or paramagnetic properties. Included among the group of molecules indirectly detectable based on light absorbance or fluorescence, for example, are various enzymes which cause appropriate substrates to convert, e.g., from non-light absorbing to light absorbing molecules, or from non-fluorescent to fluorescent molecules.

"Interferon Stimulated Response Element" refers to a short DNA sequence found in the promoter region of a gene, the expression of which is modulated by interferon.

The phrase "consisting essentially of" when referring to a particular nucleotide or amino acid means a sequence having the properties of a given SEQ ID NO:. For example, when used in reference to an amino acid sequence, the phrase includes the sequence per se and molecular modifications that would not affect the

functional and novel characteristics of the sequence.

The term "nucleic acid molecule" describes a polymer of deoxyribonucleotides (DNA) or ribonucleotides (RNA). The nucleic acid molecule may be isolated from a natural source by cDNA cloning or subtractive hybridization or synthesized manually. The nucleic acid molecule may be synthesized manually by the triester synthetic method or by using an automated DNA synthesizer.

With regard to nucleic acids used in the invention, the term "isolated nucleic acid" is sometimes employed. This term, when applied to DNA, refers to a DNA molecule that is separated from sequences with which it is immediately contiguous (in the 5' and 3' directions) in the naturally occurring genome of the organism from which it was derived. For example, the "isolated nucleic acid" may comprise a DNA molecule inserted into a vector, such as a plasmid or virus vector, or integrated into the genomic DNA of a procaryote or eucaryote. An "isolated nucleic acid molecule" may also comprise a cDNA molecule. An isolated nucleic acid molecule inserted into a vector is also sometimes referred to herein as a recombinant nucleic acid molecule.

With respect to RNA molecules, the term "isolated nucleic acid" primarily refers to an RNA molecule encoded by an isolated DNA molecule as defined above. Alternatively, the term may refer to an RNA molecule that has been sufficiently separated from RNA molecules with which it would be associated in its natural state (i.e., in cells or tissues), such that it exists in a "substantially pure" form. By the use of the term "enriched" in reference to nucleic acid it is meant that the specific DNA or RNA sequence constitutes a significantly higher fraction (2-5 fold) of the total DNA or RNA present in the cells or solution of interest than in normal or diseased cells or in the cells from which

the sequence was taken. This could be caused by a person by preferential reduction in the amount of other DNA or RNA present, or by a preferential increase in the amount of the specific DNA or RNA sequence, or by a combination of the two. However, it should be noted that "enriched" does not imply that there are no other DNA or RNA sequences present, just that the relative amount of the sequence of interest has been significantly increased.

It is also advantageous for some purposes that a nucleotide sequence be in purified form. The term "purified" in reference to nucleic acid does not require absolute purity (such as a homogeneous preparation); instead, it represents an indication that the sequence is relatively purer than in the natural environment (compared to the natural level, this level should be at least 2-5 fold greater, e.g., in terms of mg/ml). Individual clones isolated from a cDNA library may be purified to electrophoretic homogeneity. The claimed DNA molecules obtained from these clones can be obtained directly from total DNA or from total RNA. The cDNA clones are not naturally occurring, but rather are preferably obtained via manipulation of a partially purified naturally occurring substance (messenger RNA) The construction of a cDNA library from mRNA involves the creation of a synthetic substance (cDNA) and pure individual cDNA clones can be isolated from the synthetic library by clonal selection of the cells carrying the cDNA library. Thus, the process which includes the construction of a cDNA library from mRNA and isolation of distinct cDNA clones yields an approximately 10^{-6}-fold purification of the native message. Thus, purification of at least one order of magnitude, preferably two or three orders, and more preferably four or five orders of magnitude is expressly contemplated. Thus the term "substantially pure" refers to a preparation comprising

at least 50-60% by weight the compound of interest (e.g., nucleic acid, oligonucleotide, etc.). More preferably, the preparation comprises at least 75% by weight, and most preferably 90-99% by weight, the compound of interest. Purity is measured by methods appropriate for the compound of interest.

The term "complementary" describes two nucleotides that can form multiple favorable interactions with one another. For example, adenine is complementary to thymine as they can form two hydrogen bonds. Similarly, guanine and cytosine are complementary since they can form three hydrogen bonds. Thus if a nucleic acid sequence contains the following sequence of bases, thymine, adenine, guanine and cytosine, a "complement" of this nucleic acid molecule would be a molecule containing adenine in the place of thymine, thymine in the place of adenine, cytosine in the place of guanine, and guanine in the place of cytosine. Because the complement can contain a nucleic acid sequence that forms optimal interactions with the parent nucleic acid molecule, such a complement can bind with high affinity to its parent molecule.

With respect to single stranded nucleic acids, particularly oligonucleotides, the term "specifically hybridizing" refers to the association between two single-stranded nucleotide molecules of sufficiently complementary sequence to permit such hybridization under pre-determined conditions generally used in the art (sometimes termed "substantially complementary"). In particular, the term refers to hybridization of an oligonucleotide with a substantially complementary sequence contained within a single-stranded DNA or RNA molecule of the invention, to the substantial exclusion of hybridization of the oligonucleotide with single-stranded nucleic acids of non-complementary sequence. For example, specific hybridization can refer to a

sequence which hybridizes to any pregnancy specific marker gene, but does not hybridize to other bovine nucleotides. Also polynucleotide which "specifically hybridizes" may hybridize only to a pregnancy specific marker, such a pregnancy specific marker shown in Tables 2A-B. Appropriate conditions enabling specific hybridization of single stranded nucleic acid molecules of varying complementarity are well known in the art.

For instance, one common formula for calculating the stringency conditions required to achieve hybridization between nucleic acid molecules of a specified sequence homology is set forth below (Sambrook et al., Molecular Cloning, Cold Spring Harbor Laboratory (1989):

T_m = 81.5°C + 16.6Log [Na+] + 0.41(% G+C) - 0.63 (% formamide) - 600/#bp in duplex

As an illustration of the above formula, using [Na+] = [0.368] and 50% formamide, with GC content of 42% and an average probe size of 200 bases, the T_m is 57°C. The T_m of a DNA duplex decreases by 1 - 1.5°C with every 1% decrease in homology. Thus, targets with greater than about 75% sequence identity would be observed using a hybridization temperature of 42°C.

The stringency of the hybridization and wash depend primarily on the salt concentration and temperature of the solutions. In general, to maximize the rate of annealing of the probe with its target, the hybridization is usually carried out at salt and temperature conditions that are 20-25°C below the calculated T_m of the hybrid. Wash conditions should be as stringent as possible for the degree of identity of the probe for the target. In general, wash conditions are selected to be approximately 12-20°C below the T_m of the hybrid. In regards to the nucleic acids of the current invention, a moderate

stringency hybridization is defined as hybridization in 6X SSC, 5X Denhardt's solution, 0.5% SDS and 100 µg/ml denatured salmon sperm DNA at 42°C, and washed in 2X SSC and 0.5% SDS at 55°C for 15 minutes. A high stringency hybridization is defined as hybridization in 6X SSC, 5X Denhardt's solution, 0.5% SDS and 100 µg/ml denatured salmon sperm DNA at 42°C, and washed in 1X SSC and 0.5% SDS at 65°C for 15 minutes. A very high stringency hybridization is defined as hybridization in 6X SSC, 5X Denhardt's solution, 0.5% SDS and 100 µg/ml denatured salmon sperm DNA at 42°C, and washed in 0.1X SSC and 0.5% SDS at 65°C for 15 minutes.

The term "oligonucleotide," as used herein is defined as a nucleic acid molecule comprised of two or more ribo- or deoxyribonucleotides, preferably more than three. The exact size of the oligonucleotide will depend on various factors and on the particular application and use of the oligonucleotide. Oligonucleotides, which include probes and primers, can be any length from 3 nucleotides to the full length of the nucleic acid molecule, and explicitly include every possible number of contiguous nucleic acids from 3 through the full length of the polynucleotide. Preferably, oligonucleotides are at least about 10 nucleotides in length, more preferably at least 15 nucleotides in length, more preferably at least about 20 nucleotides in length.

The term "probe" as used herein refers to an oligonucleotide, polynucleotide or nucleic acid, either RNA or DNA, whether occurring naturally as in a purified restriction enzyme digest or produced synthetically, which is capable of annealing with or specifically hybridizing to a nucleic acid with sequences complementary to the probe. A probe may be either single-stranded or double-stranded. The exact length of the probe will depend upon many factors, including

temperature, source of probe and use of the method. For example, for diagnostic applications, depending on the complexity of the target sequence, the oligonucleotide probe typically contains 15-25 or more nucleotides, although it may contain fewer nucleotides. The probes herein are selected to be complementary to different strands of a particular target nucleic acid sequence. This means that the probes must be sufficiently complementary so as to be able to "specifically hybridize" or anneal with their respective target strands under a set of pre-determined conditions. Therefore, the probe sequence need not reflect the exact complementary sequence of the target. For example, a non-complementary nucleotide fragment may be attached to the 5' or 3' end of the probe, with the remainder of the probe sequence being complementary to the target strand. Alternatively, non-complementary bases or longer sequences can be interspersed into the probe, provided that the probe sequence has sufficient complementarity with the sequence of the target nucleic acid to anneal therewith specifically.

The term "primer" as used herein refers to an oligonucleotide, either RNA or DNA, either single-stranded or double-stranded, either derived from a biological system, generated by restriction enzyme digestion, or produced synthetically which, when placed in the proper environment, is able to functionally act as an initiator of template-dependent nucleic acid synthesis. When presented with an appropriate nucleic acid template, suitable nucleoside triphosphate precursors of nucleic acids, a polymerase enzyme, suitable cofactors and conditions such as a suitable temperature and pH, the primer may be extended at its 3' terminus by the addition of nucleotides by the action of a polymerase or similar activity to yield a primer

extension product. The primer may vary in length depending on the particular conditions and requirement of the application. For example, in diagnostic applications, the oligonucleotide primer is typically 15-25 or more nucleotides in length. The primer must be of sufficient complementarity to the desired template to prime the synthesis of the desired extension product, that is, to be able anneal with the desired template strand in a manner sufficient to provide the 3' hydroxyl moiety of the primer in appropriate juxtaposition for use in the initiation of synthesis by a polymerase or similar enzyme. It is not required that the primer sequence represent an exact complement of the desired template. For example, a non-complementary nucleotide sequence may be attached to the 5' end of an otherwise complementary primer. Alternatively, non-complementary bases may be interspersed within the oligonucleotide primer sequence, provided that the primer sequence has sufficient complementarity with the sequence of the desired template strand to functionally provide a template-primer complex for the synthesis of the extension product.

Polymerase chain reaction (PCR) has been described in US Patents 4,683,195, 4,800,195, and 4,965,188, the entire disclosures of which are incorporated by reference herein.

The term "vector" relates to a single or double stranded circular nucleic acid molecule that can be infected, transfected or transformed into cells and replicate independently or within the host cell genome. A circular double stranded nucleic acid molecule can be cut and thereby linearized upon treatment with restriction enzymes. An assortment of vectors, restriction enzymes, and the knowledge of the nucleotide sequences that are targeted by restriction enzymes are readily available to those skilled in the art, and

include any replicon, such as a plasmid, cosmid, bacmid, phage or virus, to which another genetic sequence or element (either DNA or RNA) may be attached so as to bring about the replication of the attached sequence or element. A nucleic acid molecule of the invention can be inserted into a vector by cutting the vector with restriction enzymes and ligating the two pieces together.

Many techniques are available to those skilled in the art to facilitate transformation, transfection, or transduction of the expression construct into a prokaryotic or eukaryotic organism. The terms "transformation", "transfection", and "transduction" refer to methods of inserting a nucleic acid and/or expression construct into a cell or host organism. These methods involve a variety of techniques, such as treating the cells with high concentrations of salt, an electric field, or detergent, to render the host cell outer membrane or wall permeable to nucleic acid molecules of interest, microinjection, PEG-fusion, and the like.

The term "promoter element" describes a nucleotide sequence that is incorporated into a vector that, once inside an appropriate cell, can facilitate transcription factor and/or polymerase binding and subsequent transcription of portions of the vector DNA into mRNA. In one embodiment, the promoter element of the present invention precedes the 5' end of the pregnancy specific marker nucleic acid molecule such that the latter is transcribed into mRNA. Host cell machinery then translates mRNA into a polypeptide.

Those skilled in the art will recognize that a nucleic acid vector can contain nucleic acid elements other than the promoter element and the pregnancy specific marker gene nucleic acid molecule. These other nucleic acid elements include, but are not limited to, origins of replication, ribosomal binding sites, nucleic

acid sequences encoding drug resistance enzymes or amino acid metabolic enzymes, and nucleic acid sequences encoding secretion signals, periplasm or peroxisome localization signals, or signals useful for polypeptide purification.

A "replicon" is any genetic element, for example, a plasmid, cosmid, bacmid, plastid, phage or virus, that is capable of replication largely under its own control. A replicon may be either RNA or DNA and may be single or double stranded.

An "expression operon" refers to a nucleic acid segment that may possess transcriptional and translational control sequences, such as promoters, enhancers, translational start signals (e.g., ATG or AUG codons), polyadenylation signals, terminators, and the like, and which facilitate the expression of a polypeptide coding sequence in a host cell or organism.

As used herein, the terms "reporter," "reporter system", "reporter gene," or "reporter gene product" shall mean an operative genetic system in which a nucleic acid comprises a gene that encodes a product that when expressed produces a reporter signal that is a readily measurable, e.g., by biological assay, immunoassay, radio immunoassay, or by colorimetric, fluorogenic, chemiluminescent or other methods. The nucleic acid may be either RNA or DNA, linear or circular, single or double stranded, antisense or sense polarity, and is operatively linked to the necessary control elements for the expression of the reporter gene product. The required control elements will vary according to the nature of the reporter system and whether the reporter gene is in the form of DNA or RNA, but may include, but not be limited to, such elements as promoters, enhancers, translational control sequences, poly A addition signals, transcriptional termination signals and the like.

The introduced nucleic acid may or may not be integrated (covalently linked) into nucleic acid of the recipient cell or organism. In bacterial, yeast, plant and mammalian cells, for example, the introduced nucleic acid may be maintained as an episomal element or independent replicon such as a plasmid. Alternatively, the introduced nucleic acid may become integrated into the nucleic acid of the recipient cell or organism and be stably maintained in that cell or organism and further passed on or inherited to progeny cells or organisms of the recipient cell or organism. Finally, the introduced nucleic acid may exist in the recipient cell or host organism only transiently.

The term "selectable marker gene" refers to a gene that when expressed confers a selectable phenotype, such as antibiotic resistance, on a transformed cell.

The term "operably linked" means that the regulatory sequences necessary for expression of the coding sequence are placed in the DNA molecule in the appropriate positions relative to the coding sequence so as to effect expression of the coding sequence. This same definition is sometimes applied to the arrangement of transcription units and other transcription control elements (e.g. enhancers) in an expression vector.

The terms "recombinant organism", or "transgenic organism" refer to organisms which have a new combination of genes or nucleic acid molecules. A new combination of genes or nucleic acid molecules can be introduced into an organism using a wide array of nucleic acid manipulation techniques available to those skilled in the art. The term "organism" relates to any living being comprised of a least one cell. An organism can be as simple as one eukaryotic cell or as complex as a mammal. Therefore, the phrase "a recombinant organism" encompasses a recombinant cell, as well as eukaryotic and prokaryotic organism.

Amino acid residues described herein are preferred to be in the "L" isomeric form. However, residues in the "D" isomeric form may be substituted for any L-amino acid residue, provided the desired properties of the polypeptide are retained. All amino-acid residue sequences represented herein conform to the conventional left-to-right amino-terminus to carboxy-terminus orientation.

Amino acid residues are identified in the present application according to the three-letter or one-letter abbreviations in the following Table:

TABLE 1

Amino Acid	3-letter Abbreviation	1-letter Abbreviation
L-Alanine	Ala	A
L-Arginine	Arg	R
L-Asparagine	Asn	N
L-Aspartic Acid	Asp	D
L-Cysteine	Cys	C
L-Glutamine	Gln	Q
L-Glutamic Acid	Glu	E
Glycine	Gly	G
L-Histidine	His	H
L-Isoleucine	Ile	I
L-Leucine	Leu	L
L-Methionine	Met	M
L-Phenylalanine	Phe	F
L-Proline	Pro	P
L-Serine	Ser	S
L-Threonine	Thr	T
L-Tryptophan	Trp	W
L-Tyrosine	Tyr	Y
L-Valine	Val	V
L-Lysine	Lys	K

The term "isolated protein" or "isolated and purified protein" is sometimes used herein. This term refers primarily to a protein produced by expression of an isolated nucleic acid molecule of the invention. Alternatively, this term may refer to a protein that has

been sufficiently separated from other proteins with which it would naturally be associated, so as to exist in "substantially pure" form. "Isolated" is not meant to exclude artificial or synthetic mixtures with other compounds or materials, or the presence of impurities that do not interfere with the fundamental activity, and that may be present, for example, due to incomplete purification, addition of stabilizers, or compounding into, for example, immunogenic preparations or pharmaceutically acceptable preparations.

By the use of the term "enriched" in reference to a polypeptide it is meant that the specific amino acid sequence constitutes a significantly higher fraction (2-5 fold) of the total of amino acid sequences present in the cells or solution of interest than in normal or diseased cells or in the cells from which the sequence was taken. This could be caused by a person by preferential reduction in the amount of other amino acid sequences present, or by a preferential increase in the amount of the specific amino acid sequence of interest, or by a combination of the two. However, it should be noted that "enriched" does not imply that there are no other amino acid sequences present, just that the relative amount of the sequence of interest has been significantly increased.

It is also advantageous for some purposes that an amino acid sequence be in purified form. The term "purified" in reference to a polypeptide does not require absolute purity (such as a homogeneous preparation); instead, it represents an indication that the sequence is relatively purer than in the natural environment (compared to the natural level this level should be at least 2-5 fold greater, e.g., in terms of mg/ml). Purification of at least one order of magnitude, preferably two or three orders, and more preferably four

or five orders of magnitude is expressly contemplated. Thus the term "substantially pure" refers to a preparation comprising at least 50-60% by weight the compound of interest (e.g., polypeptide, protein, etc.) More preferably, the preparation comprises at least 75% by weight, and most preferably 90-99% by weight, the compound of interest. Purity is measured by methods appropriate for the compound of interest (e.g. chromatographic methods, agarose or polyacrylamide gel electrophoresis, HPLC analysis, mass spectrometry and the like).

"Natural allelic variants", "mutants" and "derivatives" of particular sequences of amino acids refer to amino acid sequences that are closely related to a particular sequence but which may possess, either naturally or by design, changes in sequence or structure. By closely related, it is meant that at least about 75%, or 80% or 85% or 90% or 95%, and often, more than 90%, or more than 95% of the amino acids of the sequence match over the defined length of the amino acid sequence referred to using a specific SEQ ID NO.

Different "variants" of pregnancy specific marker members exist in nature. These variants may be alleles characterized by differences in the nucleotide sequences of the gene coding for the protein, or may involve different RNA processing or post-translational modifications. The skilled person can produce variants having single or multiple amino acid substitutions, deletions, additions or replacements. These variants may include inter alia: (a) variants in which one or more amino acid residues are substituted with conservative or non-conservative amino acids, (b) variants in which one or more amino acids are added to the pregnancy specific marker protein, (c) variants in which one or more amino acids include a substituent group, and (d) variants in

which the pregnancy specific marker protein is fused with another peptide or polypeptide such as a fusion partner, a protein tag or other chemical moiety, that may confer useful properties to the pregnancy specific marker protein, such as, for example, an epitope for an antibody, a polyhistidine sequence, a biotin moiety and the like. Other pregnancy specific marker proteins of the invention include variants in which amino acid residues from one species are substituted for the corresponding residue in another species, either at the conserved or non-conserved positions. In another embodiment, amino acid residues at non-conserved positions are substituted with conservative or non-conservative residues. The techniques for obtaining these variants, including genetic (suppressions, deletions, mutations, etc.), chemical, and enzymatic techniques are known to the person having ordinary skill in the art.

To the extent such allelic variations, analogues, fragments, derivatives, mutants, and modifications, including alternative nucleic acid processing forms and alternative post-translational modification forms result in derivatives of pregnancy specific marker genes that retain any of the biological functions of the pregnancy specific marker, they are included within the scope of this invention.

"Mature protein" or "mature polypeptide" shall mean a polypeptide possessing the sequence of the polypeptide after any processing events that normally occur to the polypeptide during the course of its genesis, such as proteolytic processing from a polyprotein precursor. In designating the sequence or boundaries of a mature protein, the first amino acid of the mature protein sequence is designated as amino acid residue 1. As used herein, any amino acid residues associated with a mature protein not naturally found associated with that protein

that precedes amino acid 1 are designated amino acid -1, -2, -3 and so on. For recombinant expression systems, a methionine initiator codon is often utilized for purposes of efficient translation. This methionine residue in the resulting polypeptide, as used herein, would be positioned at -1 relative to the mature pregnancy specific marker protein sequence.

A low molecular weight "peptide analog" or "peptidomimetic" shall mean a natural or mutant (mutated) analog of a protein, comprising a linear or discontinuous series of fragments of that protein and which may have one or more amino acids replaced with other amino acids and which has altered, enhanced or diminished biological activity when compared with the parent or nonmutated protein.

The term "tag," "tag sequence" or "protein tag" refers to a chemical moiety, either a nucleotide, oligonucleotide, polynucleotide or an amino acid, peptide or protein or other chemical, that when added to another sequence, provides additional utility or confers useful properties, particularly in the detection or isolation, of that sequence. Thus, for example, a homopolymer nucleic acid sequence or a nucleic acid sequence complementary to a capture oligonucleotide may be added to a primer or probe sequence to facilitate the subsequent isolation of an extension product or hybridized product. In the case of protein tags, histidine residues (e.g., 4 to 8 consecutive histidine residues) may be added to either the amino- or carboxy-terminus of a protein to facilitate protein isolation by chelating metal chromatography. Alternatively, amino acid sequences, peptides, proteins or fusion partners representing epitopes or binding determinants reactive with specific antibody molecules or other molecules (e.g., flag epitope, c-myc epitope,

transmembrane epitope of the influenza A virus hemaglutinin protein, protein A, cellulose binding domain, calmodulin binding protein, maltose binding protein, chitin binding domain, glutathione S-transferase, and the like) may be added to proteins to facilitate protein isolation by procedures such as affinity or immunoaffinity chromatography. Chemical tag moieties include such molecules as biotin, which may be added to either nucleic acids or proteins and facilitates isolation or detection by interaction with avidin reagents, and the like. Numerous other tag moieties are known to, and can be envisioned by the trained artisan, and are contemplated to be within the scope of this definition.

A "specific binding pair" comprises a specific binding member (sbm) and a binding partner (bp) which have a particular specificity for each other and which in normal conditions bind to each other in preference to other molecules. Examples of specific binding pairs are antigens and antibodies, ligands and receptors and complementary nucleotide sequences. The skilled person is aware of many other examples. Further, the term "specific binding pair" is also applicable where either or both of the specific binding member and the binding partner comprise a part of a large molecule. In embodiments in which the specific binding pair are nucleic acid sequences, they will be of a length to hybridize to each other under conditions of the assay, preferably greater than 10 nucleotides long, more preferably greater than 15 or 20 nucleotides long.

A "clone" or "clonal cell population" is a population of cells derived from a single cell or common ancestor by mitosis.

A "cell line" is a clone of a primary cell or cell population that is capable of stable growth *in vitro* for

many generations.

An "antibody" or "antibody molecule" is any immunoglobulin, including antibodies and fragments thereof, that binds to a specific antigen. The term includes polyclonal, monoclonal, chimeric, and bispecific antibodies. As used herein, antibody or antibody molecule contemplates both an intact immunoglobulin molecule and an immunologically active portion of an immunoglobulin molecule such as those portions known in the art as Fab, Fab', F(ab')2 and F(v).

With respect to antibodies, the term "immunologically specific" refers to antibodies that bind to one or more epitopes of a protein or compound of interest, but which do not substantially recognize and bind other molecules in a sample containing a mixed population of antigenic biological molecules. For example, immunologically specific can refer to an antibody which binds to any pregnancy specific marker polypeptide, but does not bind to other bovine polypeptides. Also an antibody which is immunologically specific may bind only to the protein expression product of a pregnancy specific marker shown in Tables 2A-B.

A "sample" or "patient sample" or "biological sample" generally refers to a sample which may be tested for a particular molecule, preferably a pregnancy specific marker molecule, such as a marker shown in Tables 2A-B. Samples may include but are not limited to cells, including uterine cells, uterine tissue, cervical tissue, chorionic villi, and body fluids, including blood, serum, plasma, urine, saliva, tears, pleural fluid and the like.

"Blood" includes but is not limited to whole blood, blood treated or mixed with anticoagulents, and any component of whole blood, including but not limited to serum, plasma, buffy coat, and purified peripheral blood

mononuclear cells.

II. Pregnancy Specific Marker Nucleic Acid Molecules, Probes, and Primers and Methods of Preparing the Same

Encompassed by the invention are isolated, enriched, or purified pregnancy specific marker nucleic acid molecules including, fragments, derivatives, mutants, and modifications of the same. Preferably, the pregnancy specific marker nucleotide is a marker shown in Table 2A.

Pregnancy specific marker polynucleotides can be any one of, or any combination of the markers shown in Tables 2A-B, and further may include variants which are at least about 75%, or 80% or 85% or 90% or 95%, and often, more than 90%, or more than 95% homologous to the markers shown in Table 2A, over the full length sequence. Pregnancy specific marker polynucleotides also may be 60% or 65% or 70% or 75% or 80% or 85% or 90% or 95% or 97% or 98% or 99% or greater than 99% homologous to the markers shown in Tables 2A-B, over the full length sequence. All homology may be computed by algorithms known in the art, such as BLAST, described in Altschul et al. (1990), J. Mol. Biol. 215:403-10, or the Smith-Waterman homology search algorithm as implemented in MPSRCH program (Oxford Molecular). Someone of ordinary skill in the art would readily be able to determine the ideal gap open penalty and gap extension penalty for a particular nucleic acid sequence. Exemplary search parameters for use with the MPSRCH program in order to identify sequences of a desired sequence identity are as follows: gap open penalty: -16; and gap extension penalty: -4.

Degenerate variants are also encompassed by the instant invention. The degeneracy of the genetic code permits substitution of certain codons by other codons,

which specify the same amino acid and hence would give rise to the same protein. The nucleic acid sequence can vary substantially since, with the exception of methionine and tryptophan, the known amino acids can be coded for by more than one codon. Thus, portions or all of the markers could be synthesized to give a nucleic acid sequence significantly different from that shown in Table 2A. The encoded amino acid sequence thereof would, however, be preserved.

In addition, the nucleic acid sequence may comprise a nucleotide sequence which results from the addition, deletion or substitution of at least one nucleotide to the 5'-end and/or the 3'-end of one or more of the markers shown in Table 2A, or a derivative thereof. Any nucleotide or polynucleotide may be used in this regard, provided that its addition, deletion or substitution does not alter the amino acid sequence which is encoded by the nucleotide sequence. For example, the present invention is intended to include any nucleic acid sequence resulting from the addition of ATG as an initiation codon at the 5'-end of the pregnancy specific marker nucleic acid sequence or its functional derivative, or from the addition of TTA, TAG or TGA as a termination codon at the 3'-end of the inventive nucleotide sequence or its derivative. Moreover, the nucleic acid molecule of the present invention may, as necessary, have restriction endonuclease recognition sites added to its 5'-end and/or 3'-end.

Such functional alterations of a given nucleic acid sequence afford an opportunity to promote secretion and/or processing of heterologous proteins encoded by foreign nucleic acid sequences fused thereto. All variations of the nucleotide sequence of the markers shown in Table 2A and fragments thereof permitted by the genetic code are, therefore, included in this invention.

Nucleic acid sequences encoding pregnancy specific markers may be isolated from appropriate biological sources using methods known in the art. In a preferred embodiment, a cDNA clone is isolated from a cDNA expression library of bovine origin. In an alternative embodiment, utilizing the sequence information provided by the cDNA sequence, genomic clones encoding a pregnancy specific marker gene may be isolated. Alternatively, cDNA or genomic clones having homology with the markers shown in Table 2A may be isolated from other species, such as mouse or human, using oligonucleotide probes corresponding to predetermined sequences within the pregnancy specific marker gene.

Nucleic acids of the present invention may be maintained as DNA in any convenient cloning vector. In a preferred embodiment, clones are maintained in a plasmid cloning/expression vector, such as pBluescript (Stratagene, La Jolla, CA), which is propagated in a suitable *E. coli* host cell. Genomic clones of the invention encoding the human or mouse pregnancy specific marker gene may be maintained in lambda phage FIX II (Stratagene).

Specific probes for identifying such sequences as the markers shown in Table 2A may be between 15 and 40 nucleotides in length.

In accordance with the present invention, nucleic acids having the appropriate level of sequence homology with the sequences encoding pregnancy specific markers may be identified by using hybridization and washing conditions of appropriate stringency as previously set forth herein.

The nucleic acid molecules described herein include cDNA, genomic DNA, RNA, and fragments thereof, which may be single- or double-stranded. Thus, oligonucleotides are provided having sequences capable of hybridizing with

at least one sequence of a nucleic acid sequence, such as selected segments of the sequences encoding pregnancy specific markers. Also contemplated in the scope of the present invention are methods of use for oligonucleotide probes which specifically hybridize with the DNA from the sequences encoding pregnancy specific markers under high stringency conditions. Primers capable of specifically amplifying the sequences encoding pregnancy specific markers are also provided. As mentioned previously, such oligonucleotides are useful for detecting, isolating and amplifying sequences encoding pregnancy specific markers.

Nucleic acid molecules encoding the oligonucleotides of the invention may be prepared by two general methods: (1) synthesis from appropriate nucleotide triphosphates, or (2) isolation from biological sources. Both methods utilize protocols well known in the art. The availability of nucleotide sequence information, such as the DNA sequences of pregnancy specific marker genes including but not limited to those listed in Table 2A, enables preparation of an isolated nucleic acid molecule of the invention by oligonucleotide synthesis. Synthetic oligonucleotides may be prepared by the phosphoramidite method employed in the Applied Biosystems 38A DNA Synthesizer or similar devices. The resultant construct may be used directly or purified according to methods known in the art, such as high performance liquid chromatography (HPLC). Long, double-stranded polynucleotides must be synthesized in stages, due to the size limitations inherent in current oligonucleotide synthetic methods. Thus, for example, a 2.4 kb double-stranded molecule may be synthesized as several smaller segments of appropriate sequence. Complementary segments thus produced may be annealed such that each segment possesses appropriate cohesive termini for attachment of an adjacent segment. Adjacent segments may be ligated by

annealing cohesive termini in the presence of DNA ligase to construct an entire 2.4 kb double-stranded molecule. A synthetic DNA molecule so constructed may then be cloned and amplified in an appropriate vector.

III. Pregnancy Specific Marker Proteins and Methods of Making the Same

Encompassed by the invention are isolated, purified, or enriched pregnancy specific marker polypeptides, including allelic variations, analogues, fragments, derivatives, mutants, and modifications of the same which retain pregnancy specific marker function. Preferably, pregnancy specific marker polypeptides include polypeptides encoded by one or more of the sequences shown in Table 2A. Pregnancy specific marker function is defined above, and includes increased expression in response to pregnancy or interferon tau, or immunological cross-reactivity with an antibody reactive with the polypeptides encoded by one or more of the sequences shown in Table 2A, or sharing an epitope with the same (as determined for example by immunological cross-reactivity between the two polypeptides.)

Pregnancy specific marker polypeptides or proteins can be encoded by one or more of the sequences shown in Table 2A, and further may include variants which are at least about 75%, or 80% or 85% or 90% or 95%, and often, more than 90%, or more than 95% homologous to the same over the full length sequence. Pregnancy specific marker polypeptides also may be 60% or 65% or 70% or 75% or 80% or 85% or 90% or 95% or 97% or 98% or 99% or greater than 99% homologous to polypeptides encoded by one or more of the sequences shown in Table 2A over the full length sequence. All homology may be computed by algorithms known in the art, such as BLAST, described in Altschul et al.(1990), J. Mol. Biol. 215:403-10, or the Smith-

Waterman homology search algorithm as implemented in MPSRCH program (Oxford Molecular). Someone of ordinary skill in the art would readily be able to determine the ideal gap open penalty and gap extension penalty for a particular protein sequence. Exemplary search parameters for use with the MPSRCH program in order to identify sequences of a desired sequence identity are as follows: gap open penalty: -12; and gap extension penalty: -2.

A full-length or truncated pregnancy specific marker protein of the present invention may be prepared in a variety of ways, according to known methods. The protein may be purified from appropriate sources, e.g., transformed bacterial or animal cultured cells or tissues, by immunoaffinity purification. Additionally, the availability of nucleic acid molecules encoding pregnancy specific markers enables production of the protein using *in vitro* expression methods known in the art. For example, a cDNA or gene may be cloned into an appropriate *in vitro* transcription vector, such as pSP64 or pSP65 for *in vitro* transcription, followed by cell-free translation in a suitable cell-free translation system, such as wheat germ or rabbit reticulocyte lysates. *In vitro* transcription and translation systems are commercially available, e.g., from Promega Biotech, Madison, Wisconsin or BRL, Rockville, Maryland.

Alternatively, according to a preferred embodiment, larger quantities of full length or truncated pregnancy specific marker polypeptides may be produced by expression in a suitable prokaryotic or eukaryotic system. For example, part or all of a DNA molecule, such as one or more of the sequences shown in Table 2A, may be inserted into a plasmid vector adapted for expression in a bacterial cell, such as *E. coli*. Such vectors comprise the regulatory elements necessary for expression of the DNA in the host cell (e.g. *E. coli*) positioned in such a

manner as to permit expression of the DNA in the host cell. Such regulatory elements required for expression include promoter sequences, transcription initiation sequences and, optionally, enhancer sequences.

The pregnancy specific marker produced by gene expression in a recombinant prokaryotic or eukaryotic system may be purified according to methods known in the art. In a preferred embodiment, a commercially available expression/secretion system can be used, whereby the recombinant protein is expressed and thereafter secreted from the host cell, to be easily purified from the surrounding medium. If expression/secretion vectors are not used, an alternative approach involves purifying the recombinant protein by affinity separation, such as by immunological interaction with antibodies that bind specifically to the recombinant protein or nickel columns for isolation of recombinant proteins tagged with 6-8 histidine residues at their N-terminus or C-terminus. Alternative tags may comprise the GST (Glutathione transferase), FLAG epitope ,or the hemagglutinin epitope. Such methods are commonly used by skilled practitioners.

The pregnancy specific marker proteins of the invention, prepared by the aforementioned methods, may be analyzed according to standard procedures. For example, such proteins may be subjected to amino acid sequence analysis, according to known methods.

IV. Pregnancy Specific Marker Antibodies and Methods of Making the Same

The present invention also provides methods of making and methods of using antibodies capable of immunospecifically binding to a protein encoded by a pregnancy specific marker, or fragments thereof. Polyclonal antibodies directed toward pregnancy specific marker proteins may be prepared according to standard

methods. In a preferred embodiment, monoclonal antibodies are prepared, which react immunospecifically with the various epitopes of the pregnancy specific marker proteins. Monoclonal antibodies have been prepared according to general methods of Köhler and Milstein, (Nature, 256: 495-7 (1975)), following standard protocols.

Purified pregnancy specific marker proteins, or fragments thereof, may be used to produce polyclonal or monoclonal antibodies which also may serve as sensitive detection reagents for the presence and accumulation of pregnancy specific marker proteins in mammalian cells. Recombinant techniques enable expression of fusion proteins containing part or all of the pregnancy specific marker protein. The full length protein or fragments of the protein may be used to advantage to generate an array of monoclonal antibodies specific for various epitopes of the protein, thereby providing even greater sensitivity for detection of the protein in cells or fluids (e.g. serum, plasma, milk, whole blood, saliva).

Antibodies according to the present invention may be modified in a number of ways. Indeed the term "antibody" should be construed as covering any binding substance having a binding domain with the required specificity. Thus, the invention covers antibody fragments, derivatives, functional equivalents, and homologues of antibodies, including synthetic molecules and molecules whose shape mimics that of an antibody enabling it to bind an antigen or epitope.

Exemplary antibody fragments, capable of binding an antigen or other binding partner, are the Fab fragment consisting of the VL, VH, C1 and CH1 domains; the Fd fragment consisting of the VH and CH1 domains; the Fv fragment consisting of the VL and VH domains of a single arm of an antibody; the dAb fragment which consists of a

VH domain; isolated CDR regions and F(ab')2 fragments, a bivalent fragment including two Fab fragments linked by a disulphide bridge at the hinge region. Single chain Fv fragments are also included.

Polyclonal or monoclonal antibodies that immunospecifically interact with pregnancy specific marker proteins can be utilized for identifying and purifying markers specific to pregnancy in the pregnancy test of the invention. For example, antibodies may be utilized for affinity separation of proteins with which they immunospecifically interact. Antibodies may also be used to immunoprecipitate proteins from a sample containing a mixture of proteins and other biological molecules. Likewise, antibodies may be used in ELISA-based or radioimmunoassay-based detection of pregnancy specific marker proteins in blood or other relevant fluids or tissues. Other uses of anti-pregnancy specific marker antibodies are described below.

V. Methods of Using Pregnancy Specific Marker Polynucleotides, Polypeptides, and Antibodies for Pregnancy Detection Assays

Pregnancy specific marker nucleic acids, including but not limited to those listed in Tables 2A-B, may be used for a variety of purposes in accordance with the present invention. Pregnancy specific marker DNA, RNA, or fragments thereof may be used as probes to detect the presence of and/or expression of pregnancy specific markers. Methods in which pregnancy specific marker nucleic acids may be utilized as probes for such assays include, but are not limited to: (1) *in situ* hybridization; (2) Southern hybridization (3) northern hybridization; (4) assorted amplification reactions such as polymerase chain reactions (PCR); 5)RNA ELISA.

The pregnancy specific marker nucleic acids of the

invention may also be utilized as probes to identify related genes from other animal species. As is well known in the art, hybridization stringencies may be adjusted to allow hybridization of nucleic acid probes with complementary sequences of varying degrees of homology. Thus, pregnancy specific marker nucleic acids may be used to advantage to identify and characterize other genes of varying degrees of relation to pregnancy specific markers, thereby enabling further characterization of pregnancy markers. Additionally, they may be used to identify genes encoding proteins that interact with pregnancy specific markers (e.g., by the "interaction trap" technique - see for example Current Protocols in Molecular Biology, ed. Ausubel, F.M., et al., John Wiley & Sons, NY, 1997), which should further accelerate identification of the components involved in pregnancy. Finally, they may be used in assay methods to detect ruminant pregnancy.

Polyclonal or monoclonal antibodies immunologically specific for proteins encoded by pregnancy specific markers or peptide fragments thereof may be used in a variety of assays designed to detect and quantitate the protein, as well as to detect ruminant pregnancy by detecting upregulation of pregnancy specific markers. Such assays include, but are not limited to: (1) flow cytometric analysis; (2) immunochemical localization of pregnancy specific markers in a body cell, tissue, or fluid; and (3) immunoblot analysis (e.g., dot blot, Western blot) (4) ELISA; (5) radioimmunoassay of extracts from various cells. Additionally, as described above, anti-pregnancy specific marker protein can be used for purification of pregnancy specific markers (e.g., affinity column purification, immunoprecipitation).

Further, assays for detecting and quantitating pregnancy specific markers, or to detect ruminant

pregnancy by detecting upregulation of pregnancy specific markers may be conducted on any type of biological sample where upregulation of these molecules is observed, including but not limited to body fluids (including blood, serum, plasma, milk, or saliva), any type of cell (such as white blood cells, uterine cells, or endometrial cells), or body tissue (such as uterine, endometrial, or any other tissue).

From the foregoing discussion, it can be seen that pregnancy specific marker nucleic acids, pregnancy specific marker expressing vectors, pregnancy specific marker proteins and anti- pregnancy specific marker antibodies of the invention can be used to detect pregnancy specific marker expression in body tissue, cells, or fluid, and alter pregnancy specific marker protein expression for purposes of assessing the genetic and protein interactions involved in pregnancy and induced expression.

In most embodiments for screening for specific marker expression associated with pregnancy, the pregnancy specific marker nucleic acid in the sample will initially be amplified, e.g. using PCR, to increase the amount of the templates as compared to other sequences present in the sample. This allows the target sequences to be detected with a high degree of sensitivity if they are present in the sample. This initial step may be avoided by using highly sensitive array techniques that are becoming increasingly important in the art.

Thus, any of the aforementioned techniques may be used to detect or quantify pregnancy specific marker expression and accordingly, determine if a ruminant is pregnant.

VI. Assays for determining ruminant pregnancy utilizing the pregnancy specific marker associated molecules of the invention.

In accordance with the present invention, it has been discovered that ruminant pregnancy is correlated with increased expression levels of certain markers, including but not limited to mRNAs and proteins. Thus, these molecules may be utilized in conventional assays to detect ruminant pregnancies. The detection of one or more of these pregnancy specific molecules in a sample is indicative of pregnancy. Alternatively, the absence of these molecules in a sample indicates that a ruminant is not pregnant.

In an exemplary method, a blood sample is obtained from a bovine suspected of being pregnant. Optionally, the blood may be centrifuged through a Hypaque gradient to obtain the buffy coat. The blood or buffy coat preparation is diluted and subjected to polymerase chain reaction conditions suitable for amplification of the pregnancy specific marker encoding mRNA. In certain applications, it may be necessary to include an agent, which lyses cells prior to performing the PCR. Such agents are well known to the skilled artisan. The reaction products are then run on a gel. An increase in pregnancy specific marker mRNA levels relative to levels obtained from a non-pregnant bovine is indicative of pregnancy in the animal being tested.

In an alternative method, uterine tissue or a chorionic villi sample is obtained from the bovine suspected of being pregnant. The cells are then lysed and PCR performed. As above, an increase in pregnancy specific marker mRNA expression levels relative to those observed in a non-pregnant animal being indicative of pregnancy in the test animal.

It is also possible to detect bovine pregnancy using

immunoassays. In an exemplary method, blood is obtained from a bovine suspected of being pregnant. As above, the blood may optionally be centrifuged through a Hypaque gradient to obtain a buffy coat. The blood or buffy coat sample is diluted and at least one antibody immunologically specific for pregnancy specific markers is added to the sample. In a preferred embodiment, the antibody is operably linked to a detectable label. Also as described above, the cells may optionally be lysed prior to contacting the sample with the antibodies immunologically specific for pregnancy specific markers. Increased production of pregnancy specific markers is assessed as a function of an increase in the detectable label relative to that obtained in parallel assays using blood from non-pregnant cow. In yet another embodiment, the blood or buffy coat preparation is serially diluted and aliquots added to a solid support. Suitable solid supports include multi-well culture dishes, blots, filter paper, and cartridges. The solid support is then contacted with the detectably labeled antibody and the amount of pregnancy specific marker protein (e.g., a protein encoded by a nucleic acid of Tables 2A-B) in the animal suspected of being pregnant is compared with the amount obtained from a non-pregnant animal as a function of detectably labeled antibody binding. An increase in the pregnancy specific marker protein level in the test animal relative to the non-pregnant control animal is indicative of pregnancy.

In another embodiment, a first antibody which binds to a first epitope on a target protein is placed in the well of a cartridge. Whole blood, blood collected in the presence of anticoagulants (e.g. sodium citrate, heparin), plasma, or serum is placed into the well of the cartridge. The target protein, if present in the sample, is bound by the first antibody, and then migrates

laterally by a wicking action, through a filter which has been sprayed with second antibody. The second antibody has affinity for a second epitope on the target protein, or alternatively for the first antibody. The second antibody is optionally labeled with a detectable label (e.g. radiolabel, gold, biotin, etc.) The second antibody localizes the antigen, and results in the appearance of a line on the filter. The first and second antibodies may be generated against the full length target protein, or against the N-terminal or C-terminal halves of the target protein, so that they recognize different epitopes of the target protein.

The foregoing immunoassay methods may also be applied to any type of sample, including a urine sample.

VIII. Kits and Articles of Manufacture

Any of the aforementioned products can be incorporated into a kit which may contain an pregnancy specific marker polynucleotide, an oligonucleotide, a polypeptide, a peptide, an antibody, a label, marker, or reporter, a pharmaceutically acceptable carrier, a physiologically acceptable carrier, instructions for use, a container, a vessel for administration, an assay substrate, or any combination thereof.

Exemplary kits contain reagents for an immunoassay such as an ELISA (e.g., detectably labeled pregnancy specific marker antibody, solid support, multiwell dish, buffer). Such a kit may optionally further comprise reagents suitable for performing polymerase chain reaction (e.g. polymerase, agarose gel, buffer).

Examples

The following examples are included to illustrate certain embodiments of the invention. They are not

intended to limit the invention in any way.

Example I

The following example sets out the methods used to create a pregnancy-specific mRNA-cDNA library. Whole blood was collected from dairy cows 18 days following artificial insemination and immediately processed to yield total cellular RNA using Tri Reagent for Whole Blood. Cows confirmed to be pregnant on day ~35 (using ultrasound) were assigned to the pregnant group. Cows found to be non-pregnant on day ~35 were assigned to the non-pregnant group. Blood RNA was pooled within pregnancy status, amplified using BD-Clontech's Smart Amplification protocol, and then converted to cDNA using RT-PCR approaches. The mRNA pools (stabilized as cDNA pools) were enriched using BD-Clontech's Subtractive Library approaches. The pregnancy-specific cDNA library (PRT) was screened using subtractive cDNA probes. Clones that were shown to be differentially expressed in 96-well dot blots were further confirmed using "virtual northern" blot. Virtual northern entailed loading either pregnancy-specific cDNAs (derived from the pregnant-specific mRNA pool) or non-pregnant-specific cDNAs (derived from the non-pregnant-specific mRNA pool) on southern blots followed by hybridization with the putative radiolabeled clones. Only clones that survived the entire analysis (subtractive library, differential hybridization and dot blot using subtractive probes, and individual confirmation of each clone using virtual northern blot) are described herein.

MATERIALS AND METHODS

RNA Isolation, CDNA synthesis and RsaI digestion:

Whole blood was collected from cows and RNA was extracted according to the Tri reagent protocol.

Briefly, 250 µl blood and EDTA were added to 750 µl Tri reagent BD, mixed and frozen. Later in the lab all 3 positive pregnant or 3 non-pregnant samples were then pooled for RNA isolation. To each 1 ml sample, 20 µl of 5 N acetic acid and 200 µl of chloroform were added and the sample was mixed. After a 10 minute room temperature incubation, each sample was centrifuged for 15 minutes at 4° C at 1200 rpm. The aqueous layer was removed and 500 µl isopropanol was added to each sample. Samples were allowed to sit at room temperature for a 10 minute room temperature incubation before being centrifuged again for 10 minutes at 4° C at 1200 rpm. The aqueous layer was removed from of each pellet and each pellet was washed with 70% ethanol before being allowed to air dry. Each pellet was resuspended in Depc treated water and quantitated. Twenty µg of each sample was sent to BD-Clontech to create a cDNA library.

PCR-Select Subtraction Procedure:

BD-Clontech PCR-Select cDNA Subtraction Kit was utilized for this procedure. (See manual - Cat. # CS1104 and CS1105). Briefly, PCR-Select cDNA subtraction was performed in both directions and subtracted cDNA libraries were prepared. Subtractive hybridization was performed with 1 (tester - PRT): 30 (driver - PRD) ratio in both directions and subtracted cDNA pool was amplified by PCR. Primary PCR samples were given 27 cycles and secondary PCR samples were given 10 cycles. Lanes contained the following: lane 1 - 1 kb Marker, lane 2 unsubtracted PRT, lane 3 - unsubtracted PRD, lane 4 - PRT primary PCR, lane 5 - PRD primary PCR, lane 6 - PRT secondary PCR, lane 7 - PRD secondary PCR and lane 8 - 1 kb marker.

Construction of Subtracted Library:

For PRT and PRD-specific subtracted cDNA pools, approximately 40 ng purified PCR-amplified secondary PCR product was cloned into the pAtlas vector (PUC base vector). The white:blue colony ratio for both the libraries is 70:30 and 90% of white colonies contained plasmid with insert. White clones were randomly selected from each library and grown in 100 µl LB- AMP (75 µg/ml) in 96 well plates. The libraries were stored in 25% glycerol at -70°C. Six plates from tester-PRT - specific (PRT 1-6) and four plates from driver-PRD-specific library (plate 1-4) were screened. Inserts from all clones were amplified and subjected to differential screening analysis. Plates were stored at -70°C with 25% glycerol.

Differential Screening of Subtracted Libraries:

Briefly, 2 µl of each PCR-amplified insert (100 ng) was arrayed in 96-well format on a nylon membrane. Two identical membranes were prepared for each 96-well plate. Each membrane was hybridized with a different P-32 labeled cDNA probe. The probe was either (1) a subtracted PRT-specific cDNA probe or (2) a subtracted PRD-specific cDNA probe.

One plate from Driver-Specific (PRD) subtracted library was subjected to differential screening analysis using the tester-specific (PRT) and driver specific (PRD)-subtracted probes. (Fig 3a)

Six plates from Tester - Specific (PRD) subtracted library was subjected to differential screening analysis using Tester-Specific (PRT) and Driver-Specific (PRD) subtracted probes. (Fig 3b)

Analysis of Differential Screening of Subtracted Libraries:

A total of 49 clones from six plates of PRT-specific subtracted library (approximately 8%), and 11 clones from one plate of PRD-specific subtracted library (about 10%) were originally determined to be differentially expressed in pregnant cows.

Virtual Northern Blot Analysis:

Northern Blot Analysis was done to confirm these data. Briefly, cDNA was amplified from Tester and Driver total RNA samples using SMART cDNA amplification technology. These cDNAs were run on an agarose gel and transferred to a membrane. Each membrane was then hybridized with different radioactive cDNA probes randomly selected from differential screening analysis. Virtual northern blot analysis on over 20 different clones from each subtracted library was performed. Six are shown here. The lanes contain the following: lane 1 SMART amplified cDNA from PRT total RNA and lane 2 SMART amplified cDNA from PRD total RNA. Results from clones from PRT-specific subtracted libraries are in fig 4a and clones from PRD-specific subtracted libraries are in fig 4b.

Sequencing:

49 clones from the PRT-specific library, and 11 clones from the PRD-specific library were sequenced. Preliminary homology analysis was done on all clones. Clones were sequenced with one of the following primers:

F1S:5′ - ATG ACG CTC AAG ACG ACA GAA - 3′ (SEQ ID NO:1)
R1S:5′ - AAA GCA GAG GTA ACA ACG CAG - 3′ (SEQ ID NO:2)

Final determination of pregnancy specific clones

The specific clones were again tested for pregnancy specificity, duplicates were eliminated, and a final list was generated. Out of the 49 cDNA clones originally described, 21 were confirmed to be specific to blood mRNA from day 18 pregnant cows (Tables 2A &B). All of these clones were from the PRT-specific library.

Further screening identified three additional clones. One of these (clone PRT6_G02) contains several 5' splice variants. These clones were amplified and cDNAs were labeled and used as probes in virtual northern blot analysis. In this analysis, mRNA was amplified from whole blood collected from day 18 pregnant or day 18 nonpregnant cows (3 cows each) using reverse transcriptase polymerase chain reaction. Resulting cDNAs representing pregnant or non-pregnant blood mRNA pools were hybridized using Southern blot with cDNA probes representing each new clone. Each of the virtual northern blots completed in this manner confirmed that the three cDNAs were up-regulated in blood mRNA from day 18 pregnant when compared to non-pregnant cows. Each clone was sequenced to determine identity.

It was also hypothesized that mRNA for each of the interferon-induced proteins (listed as clones 26-29 in Table 2B) below would be up-regulated in whole blood mRNA in day 18 pregnant when compared to nonpregnant cows. The cDNAs corresponding to each of these proteins were labeled as probes and used in the virtual northern approach described above. Each mRNA corresponding to each of these clones was up-regulated in whole blood mRNA from day 18 pregnant when compared to day 18 non-pregnant cows.

5' RACE was conducted on clones PRT6 F05 and PRT6 F07

First Strand Synthesis

One microgram of total RNA was mixed with Gene-specific primer 1 (GSP1; 2.5pmoles) in DEPC treated water to a total volume of 15.5µl, then heat denatured for 10 minutes at 70°C and chilled on ice. The following reagents were added as follows: 10X PCR buffer (final concentration 1X), 25 mM MgCl2 (final concentration 2.5mM), 10 mMdNTP mix (final concentration 0.4 mM), 0.1 M DTT (final concentration 10 mM). The mixture was mixed gently, centrifuged and incubated at 42°C for one minute before adding 1 µl of Superscript II reverse transcriptase. The incubation was continued for 50 minutes at 42°C, followed by incubation at 70°C for 15 minutes. The reaction was then placed at 37°C and 1 µl of Rnase was added for a 30 minute incubation. The cDNA was purified from the reaction by passing it over an Invitrogen S.N.A.P. column.

Tdt tailing of cDNA

An oligo dc-tail was added to all of the cDNA recovered from the S.N.A.P. column in the following reaction. The cDNA was mixed with DEPC treated water, 5 µl 5X tailing buffer, 2.5 µl 2mM dCTP for a final volume of 24 µl. The mixture was heated for 3 minutes at 94°C and placed on ice. Tdt (terminal deoxynucleotidyl transferase) was added and the reaction was mixed and placed at 37°C for 10 minutes, then heat inactivated for 10 minutes at 65°C.

First PCR

PCR was performed using Gene specific primer 2 and the abridged anchor primer provided by BD/Clontech. Both primers were used at 400 nM with 5 µl of the dc-tailed

cDNA from the previous reaction. The reaction also included 20 mM Tris (pH 7.4), 50 mM KCl, 1.5mM $MgCl_2$, 200uM each dNTP and 2.5 units Taq polymerase. Cycle parameters were as follows: preheat block to 94°C then, 94°C for 1 minute, 55°C for 1 minute and 72°C for 2 minutes for 35 cycles followed by a final extension of 72°C for 7 minutes. The PCR products were analyzed by running 10 µl each on a 1% agarose gel.

Second PCR

Because smearing appeared after the first PCR, a second PCR reaction was warranted using GSP3 (200 nM) and AUAP primer(BD/Clontech) also at 200 nM. The first PCR product was diluted 5 µl into 495 µl of TE buffer then 5 µl was used in the second PCR reaction. The remaining ingredients and cycle parameters were the same as was used for the first reaction. Again, the PCR products were analyzed by running 10 µl on a 1% agarose gel.

The cDNAs amplified using RACE were subcloned into PCR-II vector (Invitrogen) and sequenced using standard procedures (dRhodamine; ABI). The cDNA sequences were analyzed for identity to other known cDNAs using the NCBI BLAST software (University of Illinois at Urbana-Champaign and San Diego Supercomputer Center, at the University of California, San Diego University of California).

The relevant sequence information for all of the pregnancy specific marker nucleic acids is shown below. Tables 2A-B list the clones with their corresponding related genebank number and descriptor, as well as their assigned SEQ ID NO. More specific information regarding the similarity of the clones to the genebank sequences is detailed in Figure 6. More specific sequence information is described below Tables 2A-B.

Table 2A - Summary of Novel Clones Specific to Pregnancy

Clone Name	Description	Genbank Number	SEQ ID NO
PRT2_C12	Homo sapiens guanylate binding protein 1, interferon-inducible, 67kD --- AV667593 bos Taurus adipocyte cell line	gi-4503938 ref-NM_002053.1 --- gb-AV667593	5
PRT2_E08	Homo sapiens, Similar to recombining binding protein suppressor of hairless (Drosophila), clone IMAGE:4718839, mRNA	gi-18088873 gb-BC020780.1 BC020780	6
PRT3_G09	Ureaplasma urealyticum serovar 11 23S ribosomal RNA and 5S ribosomal RNA genes, complete sequence; and dihydrofolate reductase (folA) gene, partial cds	gi -14279649 gb -AF272627.1 AF272627	7
PRT5_A02	Homo sapiens B-cell CLL/lymphoma 11B (zinc finger protein) (BCL11B), transcript variant 2, mRNA	gi-12597634 ref-NM_022898.1	10
PRT5_D01	Homo sapiens, proteoglycan 1, secretory granule, clone MGC:9289	gi-15930160 gb-BC015516.1 BC015516	12
PRT6_A01	Ureaplasma urealyticum serovar 5 23S ribosomal RNA and 5S ribosomal RNA genes, complete sequence; and dihydrofolate reductase (folA) gene, partial cds. --- FM2998 Human fetal mesenchymal stem cells library Homo sapiens cDNA, MRNA sequence	gi-14279641 gb-AF272623.1 AF272623 --- FM2998 gi-11065613 gb-BF177922.1 [11065613]	14
PRT6_A04	Homo sapiens CAG-isl 7 mRNA, complete cds --- Bos taurus ovary fetus Bos taurus cDNA clone E1OV001C11 3', MRNA sequence	gi-608516 gb-U16738.1 HSU16738 --- gb-AV615795	15

PRT6_A05	Human DNA sequence from clone RP11-139H14 on chromosome 13 Contains the LCP1 (lymphocyte cytosolic protein 1 (L-plastin)) gene, part of the gene for CPB2 (carboxypeptidase B2 (plasma)), ESTs, STSs, GSSs and a CpG Island, complete sequence --- 165171 BARC 5BOV Bos taurus cDNA 5', MRNA sequence	gi-8217499 emb-AL137141.10 AL137141 --- BE480185	16
PRT6_D02	Homo sapiens, small protein effector 1 of Cdc42, clone MGC:16904 --- 336298 BARC 5BOV Bos taurus cDNA 5', MRNA sequence	gi-18043587 gb-BC012796.2 --- BG688612	17
PRT6_F05	MARC 1BOV Bos Taurus cDNA	gb-BI542085	19-23
PRT6_F08	Bos taurus clone RP42-354B6, complete sequence	gi-15011695 gb-AC091660.2	24
PRT6_G02	Homo sapiens 5#-nucleotidase, cytosolic III (NT5C3), mRNA	gi-20127662 ref-NM_016489.9	25
PRT6_F07	Homo sapiens melanoma differentiation associated protein-5 (MDA5) mRNA, complete cds. --- 24G31N1 Sus scrofa alveoli macrophage cDNA Sus scrofa cDNA, MRNA sequence	gb-AF095844 --- AW231898	26
PRT6_G10	UNKNOWN	NA: cDNA sequence below	27
PRT6_H2	Ovis Aries Secretory Protein 2EAL	gb-OAU00154	28
UBE1L	ISG15 EI, interferon induced gene	NA: cDNA and AA sequence below	29-30
PRT3_E09	Mus musculus similar to Spermidine/spermine N(1)-acetyltransferase (Diamine acetyltransferase) (SSAT) (Putrescine acetyltransferase) (LOC231081), mRNA	gi- 20824812 ref-XM_131957.1	31
PRT6_H11	Mouse DNA sequence from clone RP21-468E12 on chromosome X, complete sequence --- 204288 MARC 2BOV Bos taurus cDNA 5', MRNA sequence	gi-17017692 emb-AL163512.35 AL163512 --- BE752013	34

Table 2B - Summary of Clones Specific to Pregnancy

Clone Name	Description	Genbank Number	SEQ ID NO
PRT1_F08	Eperythrozoon wenyonii 16S ribosomal RNA gene, partial sequence	gi-2623777 gb-AF016546.1	3,4
PRT4_A12	B.taurus mRNA for LECAM-1	gi -515 emb-X62882.1 BTLECAM1	8
PRT4_F02	Homo sapiens DC6 protein (DC6), mRNA --- 1JEJ8A3.ab1 Bos taurus Jejunum #1 library Bos taurus cDNA	gi -9910185 ref-NM_020189.1 --- BM432944	9
PRT5_A07	B.taurus mRNA for Annexin I	gi-73 emb-X56649.1 BTANN1	11
PRT5_G06	Bos taurus translation initiation factor eIF-4E (eIF-4E) mRNA, complete cds	gi-7673693 gb-AF257235.1	13
PRT6_E10	Bos taurus mitochondrion, complete genome --- AU278851 Cloned bovine placenta cDNA Bos taurus cDNA clone placenta1151 3', MRNA sequence	gi-336430 gb-J01394.1 BOVMT --- AU278851	18
PRT3_F09	B.taurus mRNA for beta 2-microglobulin	gi -115 emb -X69084.1 BTB2MIT	32
PRT5_A05	Bovine Beta globin gene and globin (PSI-3) pseudogene, complete cds	gi-162740 gb-M63453.1 BOVBG	33
ISG15	ISG17, UCRP, interferon stimulated gene product 17/ubiquitin cross-reactive protein (ISG17/UCRP)	gb-AF152103, U96014,AF069133	---
1-8U	Bos taurus interferon-induced transmembrane protein 3	gb-NM_174551	35-36
Leu 13/9-27	Bos taurus interferon-induced membrane protein Leu-13/9-27	AF272042	---

Based on expression levels and other factors, preferred clones for use in the methods of the present invention are SEQ ID NO: 5, 6, 19-23, 26, 28, and 31.

Particularly preferred clones are SEQ ID NO: 5, 19-23, 26.

The sequences which are specific to bovine pregnancy are further described below.

1-F08 (SEQ ID NO:3-4)

Eperythrozoon wenyonii 16S ribosomal RNA gene, partial sequence. This sequence has polyadenylation, and a poly A tail, so this is a new protein with identity to this 16 S ribosomal RNA.

-444 bp
-DNA
-Bos taurus
1-F08 (SEQ ID NO:3)

tttttttttt tttcctaaat aaaagaactt tacaatcaga agaccttcat cgttcacgtg
gtattgctcc atcagacttt catccattgt gaaaaattcc tcactgctgc ttcccgtagg
aatatgggcc atatcccaat cccattgcgg ctgttcaacc tctcagtcca gctacccatc
actggcttgg tgggccttta ccccgccaac tacctaatag gacatattcc tatttctacg
cgaggccccc gagggagcct ctctgattta gttataaacc tatggagtat taatagtcct
ttcgaactgc tattctcctc gtaaaagtaa gttaaatatg tattactcgc ccgtttgcca
ctagtagaac aagttccact cgttcgactt gcatgtgttg tgcataccac cagcataatc
ctga

1-F08, Reverse-Complement- Sense Strand (SEQ ID NO:4)

TCAGGATTATGCTGGTGGTATGCACAACACATGCAAGTCGAACGAGTGGAACTTGTTCTACTAGTGGCAA
ACGGGCGAGTAATACATATTTAACTTACTTTTACGAGGAGAATAGCAGTTCGAAAGGACTATTAATACTC
CATAGGTTTATAACTAAATCAGAGAGGCTCCCTCGGGGGCCTCGCGTAGAAATAGGAATATGTCCTATTA
GGTAGTTGGCGGGGTAAAGGCCCACCAAGCCAGTGATGGGTAGCTGGACTGAGAGGTTGAACAGCCGCAA
TGGGATTGGGATATGGCCCATATTCCTACGGGAAGCAGCAGTGAGGAATTTTTCACAATGGATGAAAGTC
TGATGGAGCAATACCACGTGAACGATGAAGGTCTTCTGATTGTAAAGTTCTTTTATTTAGGAAAAAAAAA
AAAA

2-C12 (SEQ ID NO:5)

mRNA Homo sapiens guanylate binding protein 1, interferon-inducible, 67kDa mRNA, complete cds.
-378 bp
-DNA
-Bos taurus

gtaccagccc aaaagataaa acttttaaca tgcctcgaac tctgtatccg aaagttcttc
ccaaagaaga aatgcttcat ctttgatcga cccactcata ggaagaaact gggccagctt
gaggaactgt gtgacgatga gctggactcc gaatttgtgc aacaagctgt ggtcttctgt
tcttacatct ttagcgattc caaaactaaa gctctctcag gaggcatcaa ggtggatgga
cctcgtttag agaccctggt gcagacctat gtcaatgcca tcaacagtgg ggatctgccc
tgcatggaga atgcagtcct ggccttggct gagatcgaga actcagccgc agtacctgcc
ctggcggccg ctcgaggg

2-E08 (SEQ ID NO:6)

recombinant binding protein suppressor; Positive dot blot and virtual northern (1). No Polyadenylation

- 477 bp
- DNA
- Bos taurus

gtacgtgggg tggagttttg gcgagagttt gtggaagatg gcgcctgttg tgacaggaaa
atttggtgag cggcctccac ctaaacgact cactagagaa gctatgcgaa attatttaaa
agagcgaggg gatcaaacag tgcttattct tcatgcaaaa gttgcacaga agtcatacgg
aaatgaaaaa aggttttttt gccctcctcc ttgtgtatat cttatgggca gtggatggaa
gaaaaaaaaa gaacaaatgg aacgcgatgg ttgttcagaa caggaatctc accatgtgct
tttattggaa taggaaatag tgaccacgaa atgcagacaa ctgaacttgg aaggatcaga
aactattgca caggccaaaa acattatata tatctgattt cagacaagag aaagcacttc
atgctgtctg taaagatgtt ctatggcaac agtgatgaca ttgggtgtgt tcctaag

3-G09 (SEQ ID NO:7)

Unknown clone;

-400bp

-DNA

-Bos taurus

gtacgcgggg tagtgtttct agcgagcaga gatttttctc tctttgcttg ggactgatta
tgtgcacttg tcttaataaa agataattag gacgtgttag atatttgaca atttctagga
acaaacaaaa gaccgagtaa ataaacagaa gtaaaagaaa aacaaaagat tcttagttaa
attactaagg gctagtaacg gattccttgg aaatgatagg cgatgaagga cgtgctaatc
tgcgataagt gtcgggtagt cgataagaga ccttgatccg gcaatctccg aatgtagaaa
tacagtagaa actaatcttt ctactattgt cacctgaatc catagggtga taaggcgaac
ttggcgaagt gaaacatctc agtagccaaa aaagaaaaaa

4-A12 (SEQ ID NO:8)

LECAM-1, BTLECAM1

-371 bp

-DNA

-Bos taurus

gtaccatggc ctgtcactca ccctttgggg aacttcagct tcatgtcgca gtgtgccttc
aactgctcca agggaacaga catgattggg gttgaagaaa ccacttgtgc accatttgga
aattggtcat ctccagaacc aacctgtcga gtgattcaat gtgagcctct aacagaaccc
gatttgggaa ccatggactg taatcacccc cttgtcgact ttggcttttc ctccacatgc
accttcagtt gttcagaaga agctgagtta actggggaga aaaaagctat ctgtggattg
tctggaaatt ggtccagccc tagtccaaga tgtcaaaaaa taaacagtac ctcggccgcg
accacgctgg g

4-F2 (SEQ ID NO:9)

DC6 protein, Pos dot, VN (1)

-503 bp

-DNA

-Bos taurus

gtaccagggc tcggcctttt ggtgtgattt cagccaccaa gtcatcaaca gtaacgtgtt
ctagtccctt ttctttaatt acctctttac agtgtgcctt caactgatcc ttccagccac
attcaattaa tttagctctc agcaactctt tgaggcgttc tctttctcca gtttctatca
acttttggtt aatcgctgct ctcatctgcg catctttgtt catcttgcta accaccatca
ccgcgggcga gggccgtccc ttcccagcga cgaaatgacc cttgcgctga cgaccgttac
ctaccacact cttcggctgc ccggacctag acctccgcgt acgcggcatc tttttttctc
atggattctt aaagcctccc ttatctatcc tgttgtcctg aaaatacctg catttcttat
gtatcatatt cacacatcac tatgaatagc tagactctcc atattctata attgcttaag
atatatagtg tttaaaagta gtg

5-A2 (SEQ ID NO:10)

B Cell Lymphoma 11B; Pos dot, Pos VN1

-260 bp

-DNA

-Bos taurus

gtaccggtga gaaaaaaacc cccacatttc tgatctgtgc ttaattctct cctcccacct
ccctctggaa tggatatatt ggttggttca tatgatgtag gcacttgctg tatttttact
ggagcttgta atttttaac tgtaagcttg tccttttaaa gggatttaat gtacgtttaa
agtgtattat aacagtgtgg tagttaataa aacactattt tttttctttt gaaaaaaaaa

aaaaaaaaaa aaaaaaaaaa

5-A07 (SEQ ID NO:11)

Annexin 1, BTANN1

-455 bp

- DNA

- Bos taurus

gtacggaggt gaaaaaaaca aaaagatcct tcaaaaatgg caatggtatc tgaattcctc
aagcaggcct ggtttattga aaatgaagag caggaataca ttaaaaccgt gaaaggatcc
aaaggtggtc ctgggtcagc agtgagcccc tatcctacat tcaatccatc ctcggatgtt
gaggccttgc acaaagcaat cacagttaga ggtgtggatg aagcaaccat cattgaaatt
ctgactaaga gaaacaatgc accagcgtca gcagatcaaa gcggcctatc tgcaggagaa
ggaagccctg gatgagtctg agaagccctc ctcggtcacc tgaggagttg ttttgggctc
tattgaaact ccagcccagt ttgatgccga agagctccgt gctgccatga gggcctggga
ctgatgagac ctctgatgaa tctggcatca gaact

5-D1 (SEQ ID NO:12)

Proteoglycan 1, up dot blot only

- 480 bp

-DNA

-Bos taurus

gtacgcgggg tgtgaatcca gcagaatccc gcctccaagg actgacattt ccccactgat
gaattccaag aatttgaatg aagtcttccc tctttctgag gacatctcag gatcaggttc
tggagcagaa tctggaagtg gcttcctaaa tgaaatggaa caggaatacc aaccagtaga
agaaaacgat gctttttatt acacctttag atctaggaag agaaatgtgc cctcatacaa
ccaggacttg gggcaggatg gaccagaaga ggattttact atataaaaga gagggtttcc
catcttcaca ccaggcgatg tattttgtat accatggtta aatgattaat ttgaggacaa
aaagctttta gaaaacttaa aacatctgaa cgggaagtag tttatcatct ttttttctc
atggattctt aaaagcctcc ccttatctat cctgttgtcc tggaaaaata cctgcattct

5-G6 (SEQ ID NO:13)

Bos Taurus eIF-4E, Pos dot, VN 1&2.

-493 bp

-DNA

-Bos taurus

gtacttttt ttttttttt ttttttttg gctttcttct ctctccgagc tccgtgaatc
tctcctcctc ctcctcctat ctttacttct tgatcttcct ggcgtcgctc ttctctctcg
gctccgtgac cttcctcttc tcctgtacgc gggggactac tgaatgtgaa aacagagaaa
ctgttagaca tatagggagg gtatacaagg aaaagttagg acttcctcca aaaatagtaa
ctggttatca gtcccatgca gacacagcta ctaagcagct ccacctctaa aaataggttt
gttgtttaag aagacacctt ctgagtattc tcataggcaa ctgcctcaag caatcaagat
ttgggagctg aaggaatgcc tcttcaaaag cagaatggac tgcatttaaa tttgagttcc
atctaaatgt tgctaagata aaaggaagtt tcattagccc ttgtcttgta cctcggccgc
gaacacgcct ggg

6A01 (SEQ ID NO:14)

5 23 S ribosomal, unknown.

-501 bp

- DNA

-Bos taurus

gtacttgttc gctatcggta aatgattagt atttaggctt acccgatggt ccgggcagat
tcaaacaggg ttcacatgtc ccgccctact caggatacct ttagatctct tcatatttcg
catacggggc tttcaccctc tatggcacta cattccaata aatttctgct atagtcggag
atgcattgca aggtcctaca accccagctc aattctattg tgctggtttg gcctgttccc
cttccgctca ccactactta gggaatcact tcgttttctt ttcctttggc tactgagatg
tttcacttcg ccaagttcgc cttatcaccc tatggattca ggtgacaata gtagaaagat
tagtttctac tgtatttcta cattcggaga ttgccggatc aaggtctctt atcgataccc
gacactatcg cagatagcac gtcttcatcg cctatcattc caaggaatcg tactagccct
agtattcgcg tactgctgcg c

6-A04 (SEQ ID NO:15)

CAG-isl7 mRNA

-495 bp

-DNA

- Bos taurus

gtacttttt ttttttttt ttttttggc aacatgtcaa aaagaacttt tattctgatt
cgaatagcct cttatgcact catgctttct tgccagatgc ctttggagca ggtgctttct
gggatggagc tttctgaccc ttctgagttt taggaggagg tgctgccttc tggcctgcag
ctttctgggc aggagccttc tgggctggag ccttcttacc ctcagtggtg atctttttg
caggaacctc ggcagcagca gcagctactg cagctgcccc cttagcagca agggctttct
tgggagaagc tttcaggaga gctgcccttt gaagtttctt aacttcaatc ttgattagtc

tgttcctcat tttccttgcc ttcatgactt tgtaacgatc aaagtctgtc atcttgggct
ttcttctctc tggcttcaat cttcttgggc ccacctgtgg ctggcccccc gcgtactctg
cctgcgcgtc gaggg

6-A05 (SEQ ID NO:16)

Unknown

-493 bp

-DNA

-Bos taurus

gtacgcgggc atttaaatgt atttttaaac tctgctttaa acttctgctt ccttttttctg
tcacttttt tttaaattaa accttagctg actgacttct acagcaatat aaaggtatat
atattttcct gtatacaatt taatacatgt aaataaatta ttttctcccc tgagggcagc
agaatagtat ttgcttcttt gggttgcctt ttatgtatgt cctgtatttt taactgttat
acttctttta gaaagaaagg caaataaata taatatgtaa aggacaaggg gaaaatgaaa
attagtgaat gttcccagct ttgttaatta agttgcattc ttgaatgata gttggataag
aaatgaaata tgtttgcttt ggggaaatta ttgttgtgct aagtgtctaa ttagcatgcc
atcttgaaat tgtaatttcc tcatttctca caggactcga aagcctatta ccactgctga
acagtgctca aag

6-D02 (SEQ ID NO:17)

pos Dot, Pos VN 1, Unknown clone

-399 bp

- DNA

- Bos taurus

gtaccatcat atcagaggtg aggattcaaa catatgaatt gggaggcagc gggggaagct
gggggacata aatattcaga caacagcagg aatttatgaa ataçaagggt acattgggaa
tgtatcccta tgaccaagct tagacactga aaaactgcag gaaatacact gaaatttgaa
aaaatgagaa cctcagttac tataggtggg ggcgaaccag atggatggtg gaatcagcag
agtggacctt agttctcaca ttcctgactc ccactcccat caaacaggat gcttcctgtt
actgagctaa agagacccca gccttctcat ctgagcaggc aggagcttat ctgttctgcc
cttctaaata ccctgcaagg aagggccaga acaaaacac

6-E10 (SEQ ID NO:18)

bovine mitochondria, but has poly A tail.

-304 bp

-DNA

-Bos taurus

gtacttttt ttttttttt tttttgttgt tgagctttaa cgctttctta attgatggct
gcttttaggc ctactatggt agtgttaaat tttactctct agtcaaggtt gtatccgttt
ctaaaaggct gtacggcgta aaacgtgtta aagcaccata ccaaataggg ttaaattcta
actaagctgt aaaaagccat gattaaaata aaaataaatg acgaaagtga ccctacaata
gccgacgcac tatagctaag acccaaactg gatattaaga taccccacta tgcctaagcc
ctaa

6-F05, including splice variants (SEQ ID NO:19-23)

Marc Bos Taurus CDNA, unknown, pos dot, Vn 1&2

```
KA6/_6F5_ (19)              -------------------------GGCCACGCGTCGACTAGTACGGGG
6F5_MARC_1BOV_BI542085 (20)TGTCTTTCAGCAAGGACTGGTCTTTCTACCTGCTGTCCCACGCTGAGTT
KA8/6F5___Rev-Comp(21)      --------------------------------------------------
6F5_UW_KA_Orig_seq_(22)     --------------------------------------------------
KA5/_6F5_ (23)              --------------------------------------------------

KA6/_6F5_                   GG----GGGGGGAAGGATCAGTACAGCTGCCGAGTGAAACACGTTACTT
6F5_MARC_1BOV_BI542085      ACTCCCAACAGCAAGGATCAGTACAGCTGCCGAGTGAAACACGTTACTT
KA8/6F5___Rev-Comp          ----------------------------------------GGCCACGC
6F5_UW_KA_Orig_seq_         --------CGCCAGGGCAGGTNACAGCTGCCGAGTGAAACACGTTACTT
KA5/_6F5_                   --------------------------------------------------

KA6/_6F5_                   GGAACAACCCCGGATAGTTAAGTGGGATCGAGACCTGTAAGCAGCACCA
6F5_MARC_1BOV_BI542085      GGAACAACCCCGGATAGTTAAGTGGGATCGAGACCTGTAAGCAGCACCA
KA8/6F5___Rev-Comp          TCGACTAGTACGGGGGGGGGGGGGGATCGAGACCTGTAAGCAGCACCA
6F5_UW_KA_Orig_seq_         GGAACAACCCCGGATAGTTAAGTGGGATCNAGACCTGTAAGCAGCACCA
KA5/_6F5_                   --------------------------------------------------

KA6/_6F5_                   CGAGGTTTGAAGATGCCTCATTTGGGTTGGACTAATTCCAAATTCTGTT
6F5_MARC_1BOV_BI542085      CGAGGTTTGAAGATGCCTCATTTGGGTTGGACTAATTCCAAATTCTGTT
KA8/6F5___Rev-Comp          CGAGGTTTGAAGATGCCTCATTTGGGTTGGACTAATTCCAAATTCTGTT
6F5_UW_KA_Orig_seq_         CGAGGTTTGAAGATGCCTCANTTGGGTTGGACTAATTCCAAATTCTGTT
KA5/_6F5_                   --------------------------------------------------

KA6/_6F5_                   TCTTGCTTTTTAACACTGATAGACTTTGATGCTTTGTGCACATAAATAA
6F5_MARC_1BOV_BI542085      TCTTGCTTTTTAACACTGATAGACTTTGATGCTTTGTGCACATAAATAA
KA8/6F5___Rev-Comp          TCTTGCTTTTTAACACTGATAGACTTTGATGCTTTGTGCACATAAATAA
6F5_UW_KA_Orig_seq_         TCTTGCTTTTTAACACTGATAGACTTTGATGCTTTGTGCACATAAATAA
KA5/_6F5_                   ----------------------GGCCACGCGTCGACTAGTACGGGGG-GG
                                                   * * * * * **

KA6/_6F5_                   AAACTGTATTGATGTTACCACAAACATCTTCTTTTGATTCNCTTTGTGC
6F5_MARC_1BOV_BI542085      AAACTGTATTGATGTTACCACAAACATCTTCTTTTGATTCTCTTTGTGC
KA8/6F5___Rev-Comp          AAACTGTATTGATGTTACCACAAACATCTTCTTTTGATTCTCTTTGTGC
6F5_UW_KA_Orig_seq_         AAACTGTATTGATGTTACCACAAACATCTTCTTTTGATTCTCTTTGTGC
KA5/_6F5_                   GGGGGGTATTGATGTTACCACAAACATCTTCTTTTGACTCTCTTTGTGC
                                 ********************************** ** ********

KA6/_6F5_                   TGGTTTCTC---------------------------------------
6F5_MARC_1BOV_BI542085      TGGTTTCTCTGTAGTGTGGTCTGTGTTGGCAGTAAGCCGCAGTGGAGGT
KA8/6F5___Rev-Comp          TGGTTTCTCTG------------------------------------
6F5_UW_KA_Orig_seq_         TGGTTTCTCTGTAGTGTGGTCTGTGTTGGCAGTAAGCCGCAGTGGAGGT
KA5/_6F5_                   TGGTTTCTC---------------------------------------
                            *********
KA6/_6F5_                   --------------------------------------------------
6F5_MARC_1BOV_BI542085      CTATGCTGGCATCTTAGAGGTGTGGAGGGAAGAACTCTGACGTCCAACA
KA8/6F5___Rev-Comp          --------------------------------------------------
```

```
6F5_UW_KA_Orig_seq_      CTATGCTGGCATCTTAGAGGTGTGGAGGGAAGAACTCTGACGTCCAACA
KA5/_6F5_                ------------------------------------------------

KA6/_6F5_                ------------------------------------------------
6F5_MARC_1BOV_BI542085   CAACATCTTGGTCAAGTAAAGTCTTCAGGGTGTTTTGCGCACAAATTAT
KA8/6F5___Rev-Comp       ------------------------------------------------
6F5_UW_KA_Orig_seq_      CAACATCTTGGTCAAGTAAAGTCTTCAGGGTGTTTTGCGCACAAATTAT
KA5/_6F5_                ------------------------------------------------

KA6/_6F5_                ------------------------------------------------
6F5_MARC_1BOV_BI542085   GTCAGATAGCTATGCATGTTTAGAGTAAATCTCCAATTTGTAATTTTCC
KA8/6F5___Rev-Comp       ------------------------------------------------
6F5_UW_KA_Orig_seq_      GTCAGATAGCTATGCATGTTTAGAGTAAATCTCCAATTTGTAATTTTCC
KA5/_6F5_                ------------------------------------------------

KA6/_6F5_                ------------------------------------------------
6F5_MARC_1BOV_BI542085   TAGAATTTTGCAAAATGTTGTGACAGATATTTGTCAGAATTATTGG-AA
KA8/6F5___Rev-Comp       ------------------------------------------------
6F5_UW_KA_Orig_seq_      TAGAATTTTGCAAAATGTTGTGACAGATATTTGTCAGAATTATTGGGAA
KA5/_6F5_                ------------------------------------------------

KA6/_6F5_                ------------------------------------------------
6F5_MARC_1BOV_BI542085   TATGTTATGGTGGATGGCATTTTTTCATATAAA----------------
KA8/6F5___Rev-Comp       ------------------------------------------------
6F5_UW_KA_Orig_seq_      TATGTTATGGTGGGATGGCATTTTTTTCATATAAAATTATATTTAAGTT
KA5/_6F5_                ------------------------------------------------

KA6/_6F5_                ----------------------------------------------
6F5_MARC_1BOV_BI542085   ----------------------------------------------
KA8/6F5___Rev-Comp       ----------------------------------------------
6F5_UW_KA_Orig_seq_      CTGTTGGAGACANTGNCTAANNCCTATTTTCCATTTTTATTTTGT
KA5/_6F5_                ----------------------------------------------
```

6F-08 (SEQ ID NO:24)

Unknown bos Taurus clone RP42-354B6

-486 bp

-DNA

-Bos taurus

GTACTTTTTTTTTTTTTTTTTTTTTTCTTTTTTTTTGTAATTGTTTCTTCCTCTTCTGTTTCCCAGTAAA
TCTCTGCCCTCCATGTAATATGTTTGTAGTCCCAGTATGCCTCCTCATCATATTTAGTGCCTGACATGC
TGTGAAAAGTCCAAGGGATTAGGTCCTGTGCACACCAAGTGAAATGAGAAAACGAGAGCAAGGGACACG
TCAAGCTCACCATATATCATGGCGGAGACAGAAGAGAGATGCATAAATCTATTTGAATATAAGTTTTGT
TCTGTCACCTTGAATTAATTCTAAACAAGTTATTCTTTTTAGTAGCTGTATGCATGCTTAGTCGCTCAG
TGGTGTCCGAGTCTTTGCGACCCAAGGACTGTAGCCCACCAGGCTCCTCTCTCCATGGGATTCTCCAGG
CAGAATACTGGAGTGGGTAGCCGTCACTTCTCAGGGGATCTTCCTGACCCAGAATCAACCCGTGGGTCT
GGG

6-G02 (SEQ ID NO:25)

unknown, limited identity to NT5C3 mRNA

- 399

-DNA

- Bos taurus

gtacatggac tcttatgata ttgttctagt aaaagatgaa tcactggttg tagccaactc
tatcttacag aagattctat aaacaaccat tcttcaagaa gacctctcgc ccgtgggtgc
aattgatgaa agagctgctc atctgttcat ctggctaaaa gacttttttt atttataata
tattcttgct cctgaagttt ttcctctgta ttactgaagt attttcagat ttgttgaatc
catcaactgg aagttcctat ttctccacct ctctcaacac actcctcact atatattttt
taacagattt taaaaaattc ttagagctaa aattggaaaa ataactccct aaatttccag
aatgactttt gtagcttaat gttattatga cattttcg

6-F07 (SEQ ID NO:26)

Melanoma Differentiation Factor, AF095844, pos dot, pos VN1

TCCCTTGGTNTNTTTTANAGGCCCCTNGCGGGTTTTCCCCCCCCTTTTGATTGGGGGGTCCGATTTTTTTTTATG
CTCNTCAGGGGGGGGGGGCCCTTTTGAAAAACCCCCAGAAAGCCCCCTTTTTACGGTTCCTTGCCCTTTTGGTTG
CCCTTTTGCTCACATGTTCTTTCCTGCGTTATTCCCCTGATTNTTTGANAACCGTATTACCNCCTTTGNNTGAGC
TTATACCGCTTCCCGCAACCGAAAGACCGAGCGCAGGGGGTCANTGAGNGAGGAAGCGGAAGGGCGCCCAATAAG
CAAACCNCCTTTCCCCGGGGGTTGGCCGATTCATTAATGCAGGTGGCACGACAGGTTTCCCGAATTGAAAGCGGG
CCNTGGGGGCAAAGCAATTAATTTGAGTTAGCTCAATCATTTGGCACCCCAGGGTTTACAATTTATGCTTCCGGC
TTGTATTTTGTTTTGAAGTTTGGGCGGATAACAATTTCACACCGGAACAGNTNTGGCCATGATTACCCCAAGGTA
TTTAGGTGACAATATTGAATCTCAAGGTTTGCATCAAGGTTTGTACCGAGGTCGGAACCAATTGTAACGGCCCCC
CGTTTTGTTGAATTCGGGTTTGCCGAGGGTTGCATTGNTNNACACACACTGCAAACAATGTGTGTTGATTATCAAA
CAAATGGCGGGATAATCTGCAATAAATGTGGCCAAGCTTGGGGAACGATGATGGTGCACAAAGGCTTAGATTTGCC
TTGTCTCAAAATAAAGAATTTTGTAGTGGTTTTCCAAAATAATTTACCAAAGAAACAATACAAAAAGTGGGTAGAA
TTACCAATCACATTTCCTGATCTTAACTATTCAGAATATTGTTTGTTTAGTGATGAGGACTGACATTTGATTCAAG
ATTTCTTTTAAAAATATTATCAACTTGACATTTACATGATTTTTATTGTTTTCATCATACTACAGAACTTATGTGA
ACATTAATAAAAGTCAGTGCTTTACTCTGTATTGAGCTATATGAAAGAAGACAATATATTATGCTTTCAATTTCTA
CCTTGTTGATGGGCAGAGGTCAAAAATAAAAACCTCCCAGCAAAACCCTTTAGTGTGGAGCAGTATATTGCCGTGG
GAAGGGTGTTCGAGTATAAATAAGGATCTAAGGTACCTGC

6-G10 (SEQ ID NO:27)

Unknown clone, limited seq to human chromosome, Pos dot, Pos VN1

CAGGTACGCGGGGTTCTGACCCCAGAGGATCATAAAAGATGATGTGTGTGGTAGGTAACAATTTACAAAC
GATAAACTCCTGCACAGAAAAATGGGTAAATTGAAGTTAAACAAAAACTGAACGGCTGCCATTCATTGAC
CACTTGCCTCTGTGCTGAGTTTAGTGTGTATTATTATCTCATTTAATCATCACACCCACCCTCTGAACTA
ATTTATTCTGTTTACAGATAACGAAGCTGAGGCTTTAAGTATTTGCTAATAAATGTCAAAACTGTCCTTA
GAATCCAGGTGTTTCCTAGGCCAGTGCTCAAAACCACTGTAGAAATGGTCTTCTGTCTTAGCAATTGTAA
AGTTACTTTTAAGACCTATAGTCTTAGAAATTCTCTTTAACTGCTGACATCTTCAACTTAGGAGTGAAAA
ACACATTTGCTTCACTTAGAGTTTTGCTGCTAAGGTAGGCAGTCTTCTCAAGAATCCTCAGATTATTTCT
CTTTCATTTTTGTCTCTCTCTTGCCACTTTTTTTTTCTCGAGAGAGTCTTGTTAATGTTCGCTTCTTCTA
AAGTGTATTTATTAGTATTACCTTAGCCATCGTGCTTAATTTACATTTACGGTATTGCCCAGTCCATCAT

CATTTAAAAA

6-H2 (SEQ ID NO:28)

Unknown clone, Ovis Aries Secretory Protein 2EAL
OAU00154, Pos Dot Pos VN1

TCGCGGCCGAGGTACCCTTCCAGCGAAAAAGCACCTTCCCAAAGATTTGAATTCAGAAGGGACATGAGGG
CAGGAGCGGATAAAATCTTGCTTTCCTCTCTTTTTAAAAACTGTGGATAGCCAGAGAAGAAGAAAAGGAG
AGATAGATGGAATCAAGCTTTGTTTTTGCCCTACCCAACCACTAGACCTTCAAAGAAAGAGACATGAGCT
AGAATGAATTCCCATAAGGGTGGTATTTGTTTCTCATGGCCCTTCGAAACCACACTTAATCATACACAGT
GAGTACTCCGAAAAGGGGGAGTCCTCTGGCAAAAATGTCACTTTGCCTGCTGTGTTCAAGGCTCCCATTC
GACCCGATATTGTTAACTTTGTTCACACCAACTTGCGCAAAAACCACAGACAGCCCTATGCTGTCAGTGA
ATTAGCAGGTCATCAAACCAGTGCTGAGTCTTGGGGTACCTGCCCTGGCGGCCGCTCGA

UBE1L (SEQ ID NO:29-30)

ISG15 EI, interferon induced gene
Bovine UBE1L Nucleotide Sequence (SEQ ID NO:29)

```
   1 CGGCCGCGTC GACGAAAGCT GCCAGCTGCA TTTTCATTTC TGAGCCTTAG CTCCTGTTTG
  61 CACTGGCCAC TGCTGGGCAC TGGCTCCTGC TGGCATTGCC TCCTGTCTAC AAGCCCTGCA
 121 GCCCCGGGCC TCAGGATGGA TACCCTGGAG ACCTCCAAGT TGCTGGATAA GGAGCTATAC
 181 TCACGGCAGC TGTATGTGCT GGGCTTGCCC GCCATGCAGA GAATTCAGGG AGCCAAGGTG
 241 CTGCTGTCTG GCCTGCAGGG CCTGGGGGCT GAGGTGGCCA AAAACTTGGT CCTGATGGGC
 301 GTGGGCAGCC TCACTCTCCA TGATCCCCAC CCTACTTGCT GGTCTGACCT GGCTGCCCAG
 361 TTTCTGCTCT CAGAGCAGGA CTTGGGAAGG AGCAGAGCTG AGGCCTCTCA AAAACTTCTG
 421 GCTGAGCTCA ATGGAGCCGT TCAGGTGTCC GTCTACACAG GCGACATCAC CAAGGACCTG
 481 CTGCTGGATT TCCAGGTGGT GGTGCTGACC GCCTCGAGGC TGGAGGAGCA GCTGAGAGTG
 541 GGCACCTTGT GCCACGAGCA TGGAGTCTGC TTCCTGGTGG CAGACACCCG AGGCCTTGTG
 601 GGGCAGTTGT TCTGTGACTT TGGTGAGAAC TTCACTGTGC AGGACCCCAC AGAGGCAGAA
 661 CCCCTGACGG CCAACATCCA GCATATCTCC CAGGGCTCCC CTGGCATTCT CACTCTGAGA
 721 GAAGAGGCTG GTACCCACCA CTTCCACACA GGGGATTGGG TGACTTTCTC AGGCATTGAG
 781 GGCATGGTGG AGCTCAACGG CTGTGATCCT CGGCCTCTCC ATGTGCGGGA GGACGGGACC
 841 TTGGAGATTG GCGACACAAC AGCTTTCTCT TGTTACTTAC GTGGCGGGGC TGTCACTGAG
 901 GTCAAGAGAG CCAAGACTGT CAGCCACGAA CCCCTGGACA CAGCCCTCCT TCAACCCCGT
 961 GTGGTGGCCC AGAGTGCCCA GAAAGTTCGT GCCCGCTGCC TGCATCAGTC CTTCCGCGCA
1021 CTGCACAAGT TCCAGCAGCT CCATGGCCGC CCTCCCAAGC CTTGGGATCC TGTTGATGCA
1081 GAGATGGTGG TGGACCTGGC CCAGGCCATG GGACCACTGA AGGGGACAGA AGGAGAGCCT
1141 CTGGAAGAGC AGCTGGATGA GGCTCTGGTG CGGACAGTTG CCCTGAGCAG TGCTGGTGGC
1201 TTGAGCCCCA TGGCAGCCGT GCTGGGTGCA GTGGCTGCCC AAGAAGTGCT GAAGGCGATC
1261 TCTGGCAAGT TTATGCCCCT GGACCAGTGG CTGTACTTCG ATGCTCTGGA TTGCCTTCCA
1321 GAAGATGGGG ACCCCTTTCC CAACCCTGAG GACTGTGCAC CGAGACGCTG CCGCTATGAT
1381 GGGCAAACCG CAGTGTTCGG GACCAATTTT CAGGAGAAGC TGAGCCACCA GCACTACCTC
1441 CTGGTGGGTG CTGGCGCTGT TGGCTGCGAG CTGCTCAAAA GCTTTGCCCT GATGGGCCTG
1501 GGGGCCGGGG ACGGTGGAGG CGTGACTGTT GCCGACATGG ACCATGTGGA GCTCTCCAAC
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1561 CTCAGCCGGC AATTCCTCTT CAGGTCCCAG GACATTCATA GGAAAAAGGC AGAGGTGGCT
1621 GCAGAGGCCA CTCGCCGCCT GAATGCAGAC TTGCAGGTTA CCCCACTCAA CCTCCAGCTG
1681 GATCCCACTA CAGAGGACAT CTTTGGGGAT GACTTCTTCT CTGGTGTGAA TGGCGTGGCT
1741 GCTGCCCTGG ACACTTTTGA GGCCCGGGAC TACGTAGCTG CTCGCTGCAC CCATTTTCTG
1801 AAACCACTGC TGGAGGCGGG CACCATGGGC ACCCGGGGCA GCGCTAGCGT GTTCATACCG
1861 CACGTGACTG AGAACTACAA AGCCCCCTCT GATGCAGCTT CTGAAGATGC CCCCGACCCT
1921 GTCTGCACCG TGCGGTACAT TCCTGCCACA ACCGAGCACA CCGTGCAGTG GGCCAAGGGT
1981 GAATTTGACG ACCTTTTCTG TGAGTCTGCC AAGACCATCA ACAGCCACCC ACAGGCACTC
2041 TCGTCTCCAG AAGACTTGGT TAAGTCACAG AAGCAGCCCC TGCTGCAGAC AATGCGGGGT
2101 GTCCTGACAG AGCGTCCGCA GACCTGGCAA GACTGCGTGC TGTGGGCCTT TGGCCACTGG
2161 CAGCTCCGCT TCCATTATGG CATCACACAG CTGCTGAGGA CCTACCCACC TGACAAAGTG
2221 CAGGAGGATG GAACTCCATT CTGGTCGGGT CCCAAACAGT GTCCCCAGCC CTTGAAGTTT
2281 GATGCCAGCC AAGATATGCA CCTCCTTTAC GTGCTGGCAG CTGCCAACTT GTATGCCCAG
2341 ATGCATGGGC TGCCTGGCTC ACAAGACCAG ACTGCGCTCA GGGGACTACT GAATTTGCTG
2401 CCACTGCCTG ACCCCCAGAA CTTGGACCGC ATTTTTGCTA GTGAGCTGGA GCTGGATTCG
2461 CCTTCTGGCT GTAAGCAGCT GCATGAAGAC CTGAAGACCT GGAGCAAGGG TCCTCCCCTT
2521 AAACCCTTGA CATTTGAGAA GGACAATGAC AGCAACTTCC ACGTGGATTT TGTGGTGGCA
2581 GCGGCCAGCC TGAGAGCTCA GAACTATGGG ATCCCAGTGG CCAGCCACGC TGAGACCAAG
2641 CGAATTGTGG GCCGGATTAT CCCAGCCGTT GTCACCACTA CAGCGGCCGT GGCTGGCCTG
2701 GTGGGCCTGG AGCTGTACAA GGTGGTGGGT GGACCACGGC CCCGCCATGC CTTTCGCCAC
2761 AGCTATCTGC ACCTGGCTGA AAACTACTTT AGCCGCTGGG TACCTAAGGC CCCAGACATC
2821 CAGAAGTTCC ATCACCTGAA GTGGACCTGC TGGGACCGCC TGGAAGTGCC TGCTGGGCAG
2881 CCTGAGAGGA CCCTGGAGTC GTTGCTGGCC CACATCCAGG AGCTACAAGG ACTGAGGGTG
2941 ACGATGCTCC TGCACGGGTC GGCCCTGCTC TACTCAGCAG GATGGTCTGA GGAAAAACAG
3001 ACCCAGCACC TGTCCCGCAG GGTGACAGAT CTGGTGAAGA AGGTGCCTGG GCAGCGGGTG
3061 CTGGTATTGG AACTTGGCTA CGAGGGTGAG GAGGATGACA CTAACTTTCC ACGCTTGCAC
3121 TACAAGCTGT GACAAGGCAG CAGCCCCACC ACCCAGCTCC ATCAGGCCCT GGGTCCCGAG
3181 CCCTGCATCC CAAGCCCCCA GCAGACACTC AATAAA
```

Bovine UBE1L Protein Sequence (SEQ ID NO:30)

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   1 RPRRRKLPAA FSFLSLSSCL HWPLLGTGSC WHCLLSTSPA APGLRMDTLE TSKLLDKELY
  61 SRQLYVLGLP AMQRIQGAKV LLSGLQGLGA EVAKNLVLMG VGSLTLHDPH PTCWSDLAAQ
 121 FLLSEQDLGR SRAEASQKLL AELNGAVQVS VYTGDITKDL LLDFQVVVLT ASRLEEQLRV
 181 GTLCHEHGVC FLVADTRGLV GQLFCDFGEN FTVQDPTEAE PLTANIQHIS QGSPGILTLR
 241 EEAGTHHFHT GDWVTFSGIE GMVELNGCDP RPLHVREDGT LEIGDTTAFS CYLRGGAVTE
 301 VKRAKTVSHE PLDTALLQPR VVAQSAQKVR ARCLHQSFRA LHKFQQLHGR PPKPWDPVDA
 361 EMVVDLAQAM GPLKGTEGEP LEEQLDEALV RTVALSSAGG LSPMAAVLGA VAAQEVLKAI
 421 SGKFMPLDQW LYFDALDCLP EDGDPFPNPE DCAPRRCRYD GQTAVFGTNF QEKLSHQHYL
 481 LVGAGAVGCE LLKSFALMGL GAGDGGGVTV ADMDHVELSN LSRQFLFRSQ DIHRKKAEVA
 541 AEATRRLNAD LQVTPLNLQL DPTTEDIFGD DFFSGVNGVA AALDTFEARD YVAARCTHFL
 601 KPLLEAGTMG TRGSASVFIP HVTENYKAPS DAASEDAPDP VCTVRYIPAT TEHTVQWAKG
 661 EFDDLFCESA KTINSHPQAL SSPEDLVKSQ KQPLLQTMRG VLTERPQTWQ DCVLWAFGHW
 721 QLRFHYGITQ LLRTYPPDKV QEDGTPFWSG PKQCPQPLKF DASQDMHLLY VLAAANLYAQ
 781 MHGLPGSQDQ TALRGLLNLL PLPDPQNLDR IFASELELDS PSGCKQLHED LKTWSKGPPL
 841 KPLTFEKDND SNFHVDFVVA AASLRAQNYG IPVASHAETK RIVGRIIPAV VTTTAAVAGL
```

901 VGLELYKVVG GPRPRHAFRH SYLHLAENYF SRWVPKAPDI QKFHHLKWTC WDRLEVPAGQ
961 PERTLESLLA HIQELQGLRV TMLLHGSALL YSAGWSEEKQ TQHLSRRVTD LVKKVPGQRV
1021 LVLELGYEGE EDDTNFPRLH YKL*QGSSPT TQLHQALGPE PCIPSPQQTL NK

3-E09 (SEQ ID NO:31)

Pos dot, VN (1) This is a novel clone with low identity to known cDNAs.

-468 bp

-DNA

-Bos taurus

gtactttatt aaaaaaagaa tcaaaacaga aactctaagt acaaaagata gttccccaaa
gtttacagag tttattgccc tctaggattt gaatttgttt ttctctaaca aaacatttca
gaatgccatc tccaataact ataccaactt ctgttcctca gatgatcttt gaatcagttt
agtgattaat gggttaaata cacactctaa gtctgtattt gaatgccatg tttttagctt
aaaaaagttt acactgtgat atcttgttca aaccctcccc ccccaaaaaa aaccccacga
atccaaagtt caggaatttc tacacaatct tttccctcta aaagtttgag tgaagtcaaa
ttggatactc ttgatgaatg tgttgtgctt gtaattagaa cagaacagta actgttgctt
ttaaaaagta ttcaggccac ggcctccaat gaagacggtg aggcaaga

3-F09 (SEQ ID NO:32)

beta 2 microglobin BTB2MT

-395bp

-DNA

-Bos taurus

tacttttttt ttttttttt ttttttttag ggtttgctga agatggcggt atatagactg
tattagcaag aattggtgag gtttatcggg gtttatcgat tatagaacag gctcctctag
aaggatataa agcaccgcca agtcctttga gttttaagct gttgctagta gtactcaaga
cacccaccag aagatggaaa gccaaattac ctgaactgct atgtgtatgg gttccatcca
ccccagattg aaatcgattt gctgaagaat ggggagaaga ttaaatcgga gcagtcagac
ctgtctttca gcaaggactg gtctttctac ctgctgtccc acgctgagtt cactcccaac
agcaaggatc agtacctcgg ccgcgaacac gcggg

5-A05 (SEQ ID NO:33)

Beta Globin Gene, pos dot, pos VN1&2

-472 bp

-DNA

- Bos taurus

gtacgcgggt acacttgctt ctgacacaac cgtgttcact agcaactaca caaacagaca
ccatgctgac tgctgaggag aaggctgccg tcaccgcctt ttggggcaag gtgaaagtgg
atgaagttgg tggtgaggcc ctgggcaggc tgctggttgt ctacccctgg actcagaggt
tctttgagtc ctttggggac ttgtccactg ctgatgctgt tatgaacaac cctaaggtga
aggcccatgg caagaaggtg ctagattcct ttagtaatgg catgaagcat ctcgatgacc
tcaagggcac ctttgctgcg ctgagtgagc tgcactgtga taagctgcat gtggatcctg
agaacttcaa gctcctgggc aacgtgctag tggttgtgct ggctcgcatt ttggcaagga
atcacccgtg ctgcagctga cttcagaagg tgtgctgtgt tgcatgcctg cc

6-H11 (SEQ ID NO:34)

Unknown clone, limited seq identity to mouse chromosome, contains a poly A tail.

-299 bp

- DNA

- Bos taurus

gtacttttttt ttttttttttt ttttggggtg gaccacagag ctttttattc aaacaaagag
gtggaggctt gtgcctggcc tccctccctg cagccctcag cttgttctga catcagttgc
ccagtggtaa gtttggggtt aagcagaata tcacactggc gaccgccact atggagagaa
ggccactgtg gttgctggtg caaggccacg aggcttaggg ttgggcaggt gattcccaca
cggcccaggt gcctctcctg ggacatggtc tcatgtgtct agtgtggagg aagatgggc

ISG15

Genebank Accession AF152103, U96014, and AF069133.

1-8U (SEQ ID NO:35-36)

1-8U Nucleic Acid Sequence (SEQ ID NO:35)

ATCTGGACCGCAGTTGCTCATCTGGACTGCAGTTGCTCCGTCCCCACCATGAACCGC
ACATCCCAGCTCTTACTCACTGGGGCCCACGGGGCGGTGCCCCCAGCCTATGAGGTG
CTCAAGGAGGAGCACGAGGTGGCCGTGCTGGGGGCGCCCCAGAGCCAGGCGCCCCTG
ACGACCACGGTGATCAACATCCGCAGCGACACCGCCGTGCCCGACCACATCGTGTGG
TCCCTGTTCAACACCATCTTCATGAACTGGTGCTGCCTGGGCTTCGTGGCATTCGCC
TACTCTGTGAAGTCTAGGGACCGGAAGATGGTCGGCGACATCACTGGGGCCCAGAGC
TACGCCTCCACCGCCAAATGCCTGAACATCTGCTCCCTGGTCCTGGGCATCCTTCTG
ACTGTCGTCCTCATCGTCCTCGTGTCCAACGGCTCCCTGATGATCGTTCAAGCAGTC

TCCGAGCTCATGCAAAACTACGGAGGCCACTAGGCCTGCCCAAAAGCCCGAGGCAGT
CGCCCCTTTCCCCGCAGCCTATCCAGGCACCTGCCCCCGTGAAATAAAAGGAGGGTT
TGTGTGTTG

1-8U Protein Sequence (SEQ ID NO:36)
MNRTSQLLLTGAHGAVPPAYEVLKEEHEVAVLGAPQSQAPLTTTVINIRSDTAVPDH
IVWSLFNTIFMNWCCLGFVAFAYSVKSRDRKMVGDITGAQSYASTAKCLNICSLVLG
ILLTVVLIVLVSNGSLMIVQAVSELMQNYGGH

Leu 13/9-27

Genebank Accession AF272042

While certain of the preferred embodiments of the present invention have been described and specifically exemplified above, it is not intended that the invention be limited to such embodiments. Various modifications may be made thereto without departing from the scope and spirit of the present invention, as set forth in the following claims.